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# **Liver Transplantation**



**A CURRENT PROBLEMS  
IN SURGERY® CLASSIC**

**Liver Transplantation  
A 31-Year Perspective**

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To Joy and Jackie



## FOREWORD

Occasionally in medicine a single individual makes a contribution of such magnitude and significance that it clearly represents a new direction in the field. Such is the case in this monograph devoted to transplantation of the liver which originally appeared in **Current Problems in Surgery**. Dr. Thomas Starzl and his colleagues, formerly of the University of Colorado and now of the University of Pittsburgh, have, by their many seminal contributions, had a great influence on the entire field of transplantation; but it is transplantation of the liver which has gained these scientists their widest recognition.

The operation began as an idea only 30 years ago and the seemingly painful and slow steps which subsequently led from early clinical trials to the current stage of development are remarkable. Today, the procedure is performed in a number of medical centers around the world, in all age groups of patients, and for a wide variety of indications—a tribute to the remarkable efforts and the persistence of Dr. Starzl and his group.

In this volume, Dr. Starzl and Dr. Demetris cover all aspects of hepatic transplantation, including the technical points of the replacement operation, the prevention of rejection, and the complications both of the operation and of the postoperative immunosuppressed state. In the closing parts of this treatise, the authors review the newly emerging technique of multiple organ transplantation, auxiliary transplantation, and the practical limitations of the procedure, including organ donation and economic factors.

This contribution is authoritative and excellent, and will surely become a classic in the field.

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## ACKNOWLEDGMENTS

We wish to thank Mrs. Terry Mangan for managing the Pittsburgh editorial duties. Her ever-pleasant attitude, diligence and persistence kept the authors "on track," when it was easy to lose enthusiasm. Without her contributions, it is likely the text would not have been completed. We are especially grateful for her ability to locate figures and references given only "free associations" and her willingness to persevere at odd hours, after her duties as a home manager had begun.

*Thomas E. Starzl, M.D., Ph.D.*  
*Anthony J. Demetris, M.D.*



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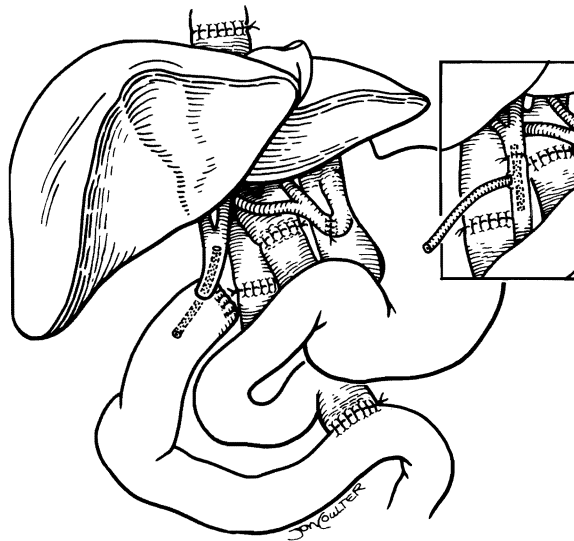
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# INTRODUCTION

Liver transplantation has had intellectual as well as practical ramifications in all aspects of hepatology. In 1955, when the concept of transplanting a whole liver was first mentioned in the medical literature,<sup>1</sup> the specialty of hepatology still had ambiguous boundaries and purposes. This account will show how such a seemingly fanciful idea as transplanting a liver became a practical reality and thereby helped shape the specialty of hepatology during the succeeding 30 years. During the same period, transplantation fostered changes in practically every aspect of hepatology and liver surgery to the extent that it is no longer possible to have a liver disease center without hepatic transplant capability.



**FIG 1.**

Orthotopic liver transplantation (liver replacement). Biliary tract reconstruction usually is with choledochojejunostomy (to a Roux limb) or (*inset*) with a choledochocholedochostomy, which is stented with a T tube.

The liver can be transplanted as an extra (auxiliary) organ at an ectopic site or in the normal (orthotopic) location after removal of the host liver (Fig 1). This review will be preoccupied with the orthotopic procedure. However, there has been renewed interest in the auxiliary operation, which will be discussed separately at the end of this monograph. In addition to the potential clinical value of auxiliary hepatic transplants, efforts to define the optimal way of revascularizing auxiliary liver grafts opened a new field of physiologic and biochemical research by demonstrating that splanchnic venous blood possesses specific liver supporting (hepatotrophic) qualities.<sup>2,3</sup>

As we develop the subject of orthotopic transplantation, we will provide a running historical perspective since even the earliest major publications on this subject are less than 30 years old, and many are still of current interest. However, particular attention will be paid to the massive literature that has developed since June 1983, when the conclusion was reached by a Consensus Development Conference that orthotopic liver transplantation had become a service as opposed to an experimental procedure.<sup>4</sup>

## DEVELOPMENT OF THE REPLACEMENT OPERATION

The distinction between creative and delusional thinking usually becomes clear only in retrospect. The idea of liver replacement first surfaced in 1956 with a publication by Dr. Jack Cannon, who was working at the new Department of Surgery, University of California, Los Angeles (UCLA).<sup>5</sup> Because it was suspected at that time that the liver might play a role in rejection, Cannon apparently hoped that a hepatic homograft would be more kindly received than other transplanted organs since presumably it would not contribute to its own destruction. There was no journal devoted to transplantation in 1956, and abstracts or brief articles in this field were published in an appendix to *Plastic and Reconstructive Surgery*, which was called *Transplantation Bulletin*. Cannon's article in *Transplantation Bulletin* was less than one page long. It did not have a title, and descriptions of the procedure or even of the animal species used were omitted. Cannon referred to "several successful operations" but without survival of the recipients.

Even for nonhistorians, Cannon's one-page article may have the special fascination of a solitary dot on a nearly empty canvas on which a complex mural was to quickly and unexpectedly appear. There was no identifiable reason in 1956 to hope that any whole organ could be transplanted successfully, including the kidney, much less more complicated grafts such as the liver, heart, or lung.

The most unattainable ingredient of potential success was prevention of rejection of transplanted tissues and organs. The seeming insurmountability of this biologic barrier undoubtedly discouraged research efforts at liver replacement. Nevertheless, the technical feasibility of liver transplantation was to be tested in dogs with increasing conviction and determination by independent teams in Boston<sup>6,7</sup> and Chicago,<sup>8,9</sup> beginning in the summer of 1958. The canine model proved to be a difficult one technically, and systematic investigation of liver replacement was hampered seriously by the fact that this operation could be done successfully in only a few laboratories in the world. In recent times, improvements in the clinical operation have been incorporated into the dog procedure.<sup>10, 11</sup>

The technical requirements for liver transplantation in dogs are almost too complex for simple categorization. However, two cardinal requirements for perioperative survival emerged from this work more than 30 years ago. The first was adequate preservation of the homograft during its procurement and the period of devascularization.<sup>8</sup> The second was decompression with veno-venous bypasses of the obstructed recipient splanchnic and systemic venous beds during the anhepatic period when the host liver was being removed and the new liver was being inserted.<sup>6, 8</sup>

We will consider these principles as they have been applied clinically under the general headings of donor and recipient operations and then discuss the more conventional surgical components of liver transplantations including recipient hepatectomy, graft revascularization, biliary tract reconstruction, and hemostasis.

#### *DONOR HEPATECTOMY AND INITIAL COOLING*

##### *Hypothermia and Core Cooling*

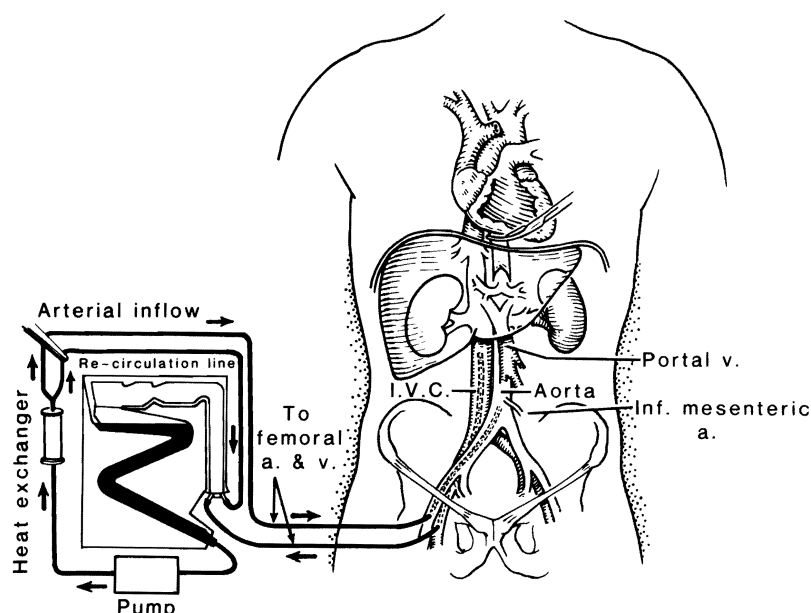
Steps in the development of liver graft procurement and preservation have been few. However, these steps have had an importance beyond their application for liver replacement, since the principles involved are germane to the preservation of other whole organs. The first innovation of core cooling by infusion of chilled lactated Ringier's solution into the portal vein may have been the most important.<sup>8</sup> Before core cooling was used, survival of dogs after liver transplantation was virtually never obtained, but afterward, success became almost routine.<sup>8</sup>

At an even earlier time, it was appreciated by cardiac surgeons that hypothermia protected ischemic tissues and organs below the level of aortic<sup>12</sup> and renal pedicle<sup>13</sup> crossclamping. To our knowledge, Lillehei and associates were the first to use hypothermia in transplantation.<sup>14</sup> They immersed dog intestinal grafts in iced saline before autotransplantation or homotransplantation. Later, the extent of hypothermic protection from ischemia was quantified by Sicular and Moore, who reported that enzyme degradation in hepatic slices was greatly slowed by refrigeration.<sup>15</sup>

The cooling of organs with fluids infused via the vascular system was such an obvious expedient that failure to do it can only be described as surprising. Even more inexplicable was failure to core cool kidney transplants. This was not done until long after the initial research with canine liver transplantation had been completed. Then, as a direct result of our experience with the dog livers, we introduced core cooling of kidney grafts into clinical practice. At first, we had protected human renal homografts by inducing total body hypothermia of living volunteer donors, but before long, we replaced

this cumbersome and potentially dangerous method with infusion of chilled fluid into the kidney immediately after its removal.<sup>16</sup>

Today, core cooling is the first step in the preservation of all whole organ grafts, and it is most often done with the organs in place by some variant of the in situ technique originally described by Marchioro and associates.<sup>17</sup> These investigators used a heart-lung apparatus that contained a heat exchanger to cool the carcass of dogs before beginning organ removal and to maintain hypothermic perfusion thereafter. This method (Fig 2) for the immediate or continuous in situ hypothermic perfusion of cadaveric livers and kidneys was used clinically long before the acceptance of brain death conditions<sup>18</sup>; the technique has had a renaissance recently for procurement of thoracic organs.<sup>19, 20</sup> Ackerman and Snell<sup>21</sup> and Merkel and col-



**FIG 2.**

First technique of in situ cooling by extracorporeal hypothermic perfusion. The catheters were inserted via the femoral vessels into the aorta and vena cava as soon as possible after death. Temperature control was provided with a heat exchanger. Crossclamping of the thoracic aorta limited perfusion to the lower part of the body. This method of cadaveric organ procurement was used from 1962 to 1969, before the acceptance of brain death. The preliminary stages of this approach provided the basis for subsequent in situ infusion techniques. (Redrawn from Starzl TE: *Experience in Renal Transplantation*. WB Saunders Co, Philadelphia, 1964.)

leagues<sup>22</sup> popularized much simpler methods of in situ cooling of cadaveric kidneys with cold electrolyte solutions infused into the distal aorta.

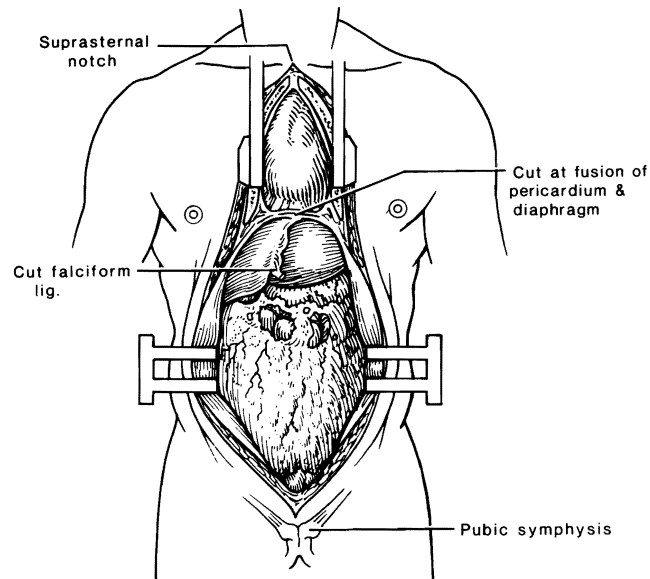
### ***Core Cooling for Multiple-Organ Procurement***

An extension of these primitive in situ cold infusion techniques has allowed removal of all thoracic and abdominal organs, including the liver, without jeopardizing any of the individual organs.<sup>23</sup> The techniques of organ procurement and preservation used clinically came from the laboratory procedures as described earlier. However, much further development was required for the procurement of multiple organs from human cadaveric donors that were expected to provide kidneys, hearts, pancreases, and other tissues as well as livers.

In the first trials of multiple-organ procurement, in situ cooling was not used. The individual organs were skeletonized, and after all of the dissection was completed, the kidneys were removed and cold perfused on the back table. At a second stage, the liver and heart were removed simultaneously. The removal of all four organs was a rare event, and the first time the kidneys, liver, and heart were removed from a single donor was on April 17, 1978 during a visit by the University of Colorado team to the University of Minnesota.

It quickly became obvious that in situ cooling of organs was going to be necessary if extrarenal organ transplantation were to flourish. During the times when the numbers of liver or heart transplants were small, the annoyance caused for renal transplant surgeons by multiple-organ procurement was relatively minor. As multiple-organ procurement became routine, a major educational effort was required to recruit the cooperation of kidney transplanters. The in situ procedures were developed in Denver, and when the Colorado team moved to Pittsburgh, these were demonstrated throughout the eastern two thirds of the United States. At the request of the Surgeon General of the United States, Dr. C. E. Koop, a description of the new operation of multiple-organ procurement was published.<sup>23</sup> Modifications of this procedure have been made for unstable donors and even for donors whose hearts have ceased to beat.<sup>24</sup> In less than 5 years, multiple-organ procurement, using techniques that are interchangeable not only from city to city but from country to country, had become standardized in all parts of the world.

A complete midline abdominal and thoracic incision is made (Fig 3). The aorta at the diaphragm is encircled so that it can be cross-clamped when the core cooling is begun. The distal aorta is used as an entry site for the fluid infusion (Fig 4). By coordination of the fluid infusion and the crossclamping of the great vessels and by dissection and ligation of appropriate arterial branches, the cold infusate



**FIG 3.**

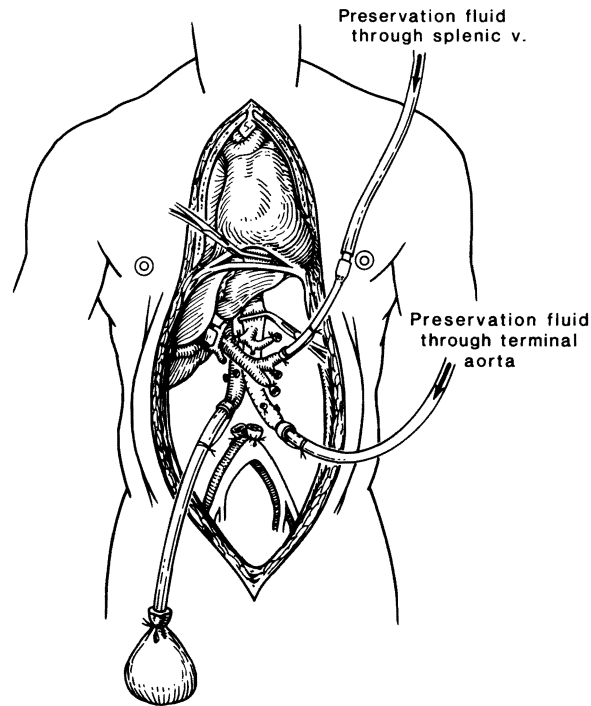
Total midline incision used for cadaveric donors. (Redrawn from Starzl TE: *Surg Gynecol Obstet* 1984; 158:223–230.)

can be made to go selectively to those organs (including the liver) that are to be used (see Fig 4). The portal vein of the liver also is infused after a catheter is placed into it through the splenic vein or other major tributary (see Fig 4). Core cooling of the thoracic organs is accomplished with the same principles.<sup>23</sup>

There is little point in providing further details of the donor operation. Those interested in procurement procedures should study the description of the originally described technique<sup>23</sup> or the derivative method called the *rapid flush technique*,<sup>24</sup> which can be used for unstable donors or even for donors who develop a cardiac arrest (see the next section). With the rapid flush method, almost no dissection is performed initially. The organs are quickly chilled and washed free of blood in situ by aortic infusion and infusion through a distal portal branch such as the inferior mesenteric vein. They can then be removed swiftly in a bloodless field.

#### ***Liver Procurement In Non-Heart-Beating Donors***

When liver transplantation was first performed experimentally and clinically, it was thought that the liver would be exquisitely sensitive to warm ischemia.<sup>8</sup> This perception has changed in the ensuing



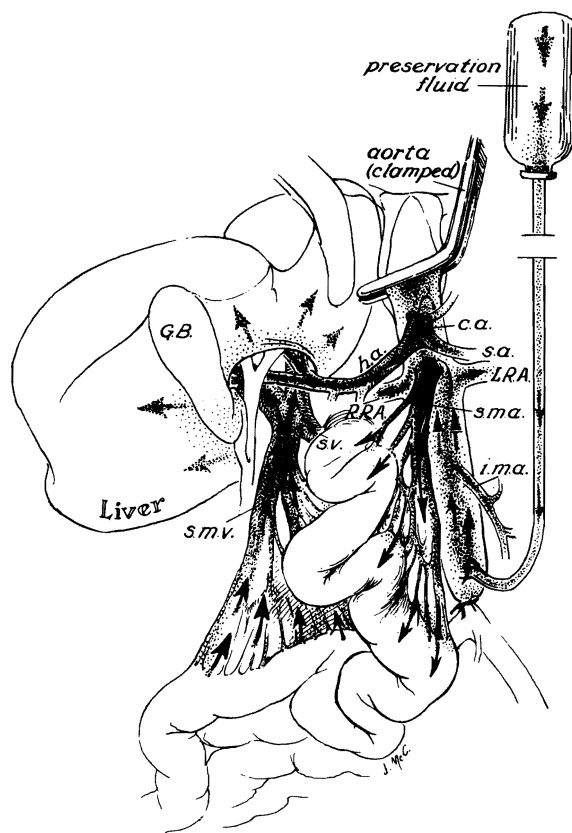
**FIG 4.**

Principle of in situ cooling used for multiple organ procurement. With limited preliminary dissection of the aorta and of the great splanchnic veins (in this case the splenic vein), cold infusates can be used to chill organs in situ. In this case, the kidneys and liver were to be removed. Note the aortic crossclamp above the celiac axis. (From Starzl TE, Hakala TR, Shaw BW Jr, et al: *Surg Gynecol Obstet* 1984; 158:223–230.)

years, particularly with the demonstration by Huguet and associates that the human liver can tolerate at least 1 hour of warm ischemia with relative impunity.<sup>25</sup> Studies in normal dogs have shown that the portal triad usually can be crossclamped for at least 2 hours without mortality, providing there is perfect decompression of the obstructed portal venous drainage.<sup>26</sup>

If a cardiac arrest occurs in a patient considered to be a good donor, it is possible to quickly open the abdomen, encircle the proximal aorta at the level of the diaphragm, and cannulate the terminal abdominal aorta or one of the iliac arteries (Fig 5). Within 5 or 10 minutes, core cooling can be started with an infusion of cold solution. The aorta is crossclamped near the diaphragm. The inferior vena cava is decompressed by incising it. The liver becomes blanched and free of blood with surprising rapidity. Within 2 or 3



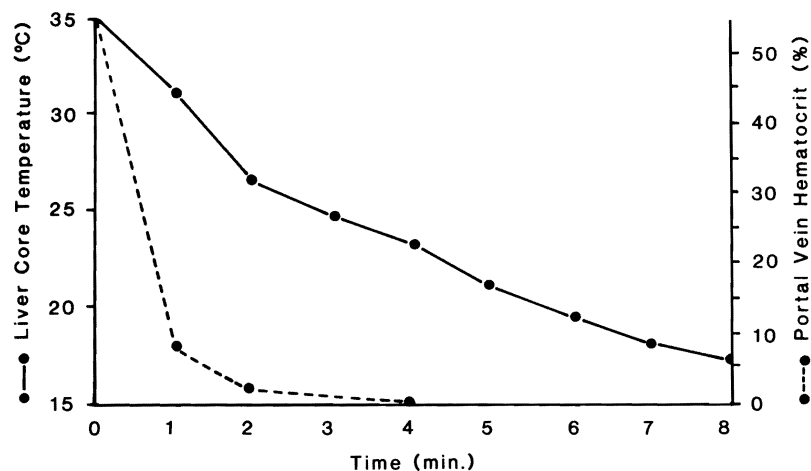


**FIG 5.**

If there is not time to insert a splanchnic venous catheter for infusion, the rapid infusion of cold fluid into the aorta alone will promptly cool the liver since the superior mesenteric venous blood contributes to the hepatic cooling (see Fig 6). All that is necessary is to insert the catheter into the distal aorta and to crossclamp the aorta at the diaphragm. (From Starzl TE, Iwatsuki S, Shaw BW Jr, et al: *Transplant Proc* 1985; 17:250–258. Used by permission.)

minutes, the liver becomes palpably cold. At the same time, the intestines become blanched from the superior mesenteric artery infusion, and blood in the portal vein that has passed through the splanchnic capillary bed becomes clear and hemoglobin free (Fig 6). Thus, full perfusion of the liver eventually is assured even though the chilled fluid is instilled only into the aorta (see Figs 5 and 6).

In adults, 2 or 3 L of cold solution rapidly infused into the distal aorta are required to bring the liver into a cryoprotective range of less than 28°C. After this has been achieved, the rest of the procure-



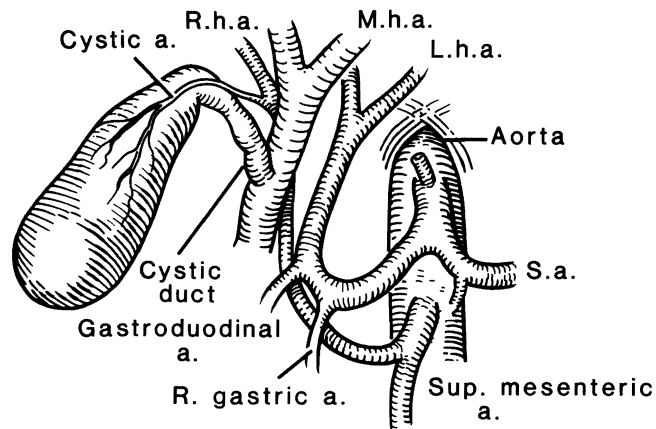
**FIG 6.**

Core cooling of the liver with aortic infusion alone. Note that the body (and liver) temperature becomes cryoprotective within 2 or 3 minutes after beginning the aortic infusion. The hematocrit of the portal venous blood is quickly diluted, meaning that the liver is being perfused with the increasingly asanguinous cold blood returning from the splanchnic venous bed.

ment can be carried out at a more leisurely pace. This is facilitated by the fact that there is now a bloodless field. We have used this technique to recover satisfactory livers from many donors with absent or ineffective heartbeat.<sup>27</sup> The method has been used with considerable success in Sweden, which did not have "brain death" laws until recently.<sup>28</sup> Our experience and that of the Swedish workers under these circumstances have been almost as good as with the standard procurements in cadaveric donors with beating hearts. However, a high level of skill is required to prevent the loss of these organs, and discriminating judgment is necessary about which organs have a good chance of being satisfactory. Only surgeons experienced in procurement of donor organs will be capable of this kind of work.

#### *Donor Anomalies*

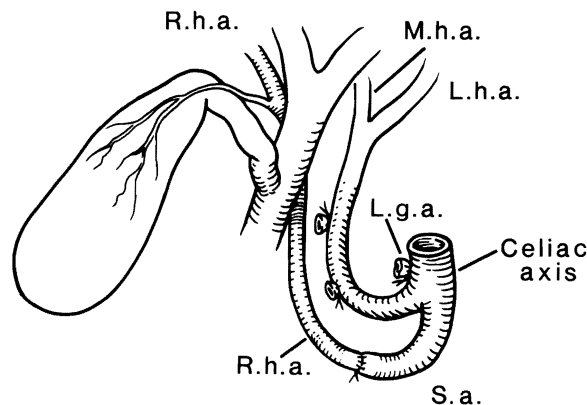
In at least one third of the human donors, arterial anomalies will be encountered whereby some or all of the liver is supplied by branches of the left gastric artery, superior mesenteric artery, or direct branches from the aorta instead of by ramifications of the common or proper hepatic artery (Fig 7). Special techniques that allow essentially all such livers to be used have been developed.<sup>29-35</sup> Most



**FIG 7.**

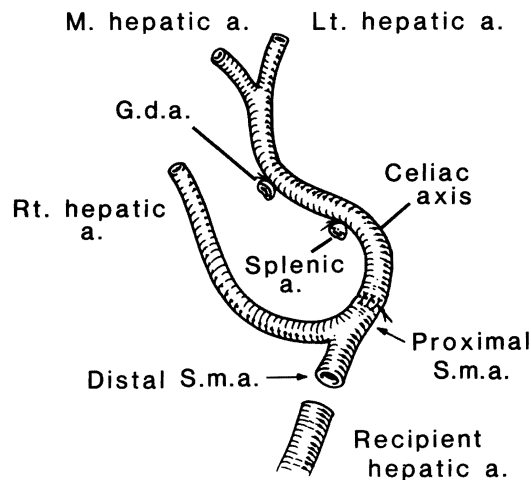
A common anomaly in which a right hepatic artery originates from the superior mesenteric artery. This right artery always is posterior to the portal vein.

of these techniques have in common the conversion of multiple vessels into a single trunk by back-table dissection and anastomoses (Figs 8–10). Uniting the celiac axis and superior mesenteric artery as shown in Figure 10 may leave an excessively long vessel and a bulge at the site of the fold-over anastomosis. Consequently, if this princi-



**FIG 8.**

With the anomaly in Figure 7, the splenic artery can be anastomosed to the anomalous right hepatic artery, thereby converting the origin of the blood supply to a single vessel based on the celiac axis. (Redrawn from Starzl TE: *Experience in Hepatic Transplantation*. Philadelphia, WB Saunders Co, 1969.)



**FIG 9.**

Alternative methods to reconstruct the anomaly shown in Figure 7. The proximal (left) or distal (right) end of the superior mesenteric artery containing the anomalous right hepatic artery is anastomosed to the graft celiac axis. The open end is sewed to the recipient celiac axis.

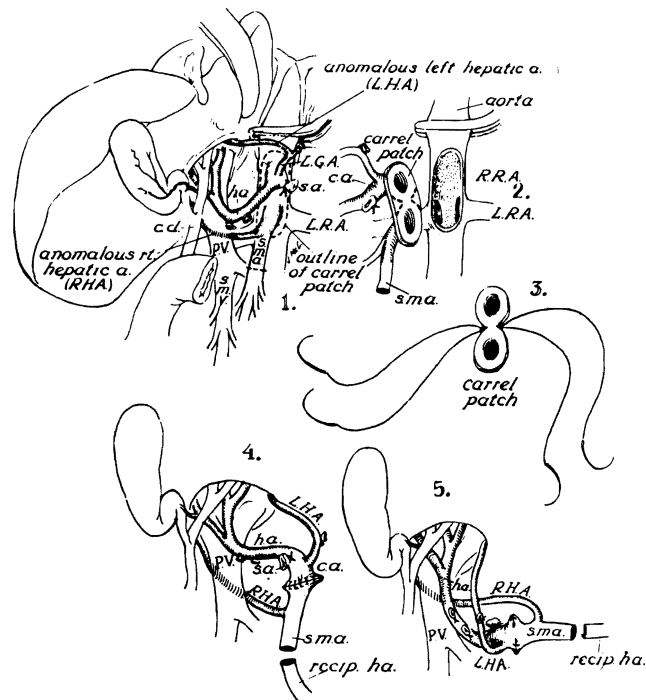
ple is employed, an end-to-end celiac axis to superior mesenteric artery anastomosis may be preferable (see Fig 8 or 9).

#### ***Vascular Homografts***

An integral part of the donor operation should be procurement of free grafts of the iliac arteries and veins, since these can be used to reconstruct anomalies or damaged vessels of livers (Fig 11), kidneys, and other organs. At the time of procurement, these grafts are placed in a solution developed at the University of Wisconsin (UW solution), where they can be used for at least 1 or 2 days or possibly longer. When arterial grafts are needed, they are usually based below the recipient renal arteries. They can be brought anterior to the pancreas (Fig 12) or behind the pancreas through tunnels created by finger dissection (Fig 13). Vein grafts will be discussed later.

#### ***MEANS OF SUBSEQUENT PRESERVATION***

In dogs, the liver can be transplanted successfully as long as 6 to 12 hours after cooling with lactated Ringer's solution and storage at 4°C.<sup>36</sup> Further extension of this period and improvement of safety have depended on one of two prototype strategies, derived from research done mainly with kidneys and applied secondarily to livers.

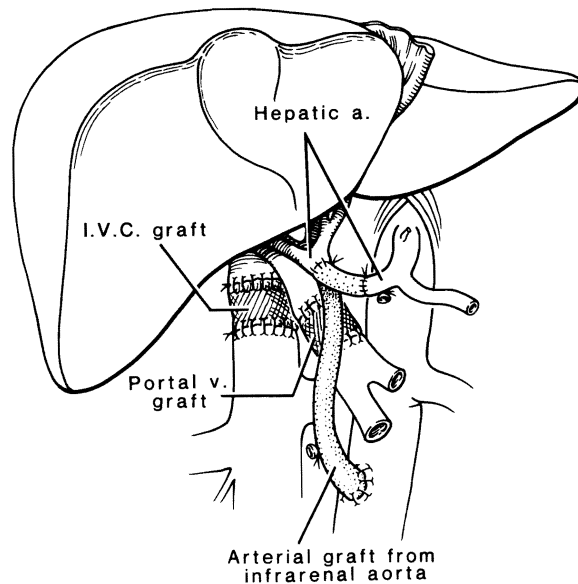


**FIG 10.**

Same principle as the reconstruction of Figure 9 (left). However, the origin of the superior mesenteric artery and celiac axis are folded together, leaving the distal end of the superior mesenteric artery for anastomosis to the recipient. There is a small left hepatic artery in this case originating from the left gastric artery. This latter anomaly is very commonly found in association with a right hepatic artery of superior mesenteric arterial origin. (From Gordon RD, Shaw BW Jr, Iwatsuki S, et al: *Surg Gynecol Obstet* 1985; 160:474–476. Used by permission.)

### ***Ex Vivo Perfusion After Initial Cooling***

With one approach, a continuous circulation has been provided with a cold perfusate primed with blood and oxygenated within a hyperbaric oxygen chamber.<sup>37</sup> This method, which originally was used for kidneys by Ackerman and Barnard,<sup>38</sup> has permitted the successful preservation of dog livers for as long as 2 days<sup>37</sup> and was applied clinically with remarkable success in several human cases in the pre-brain death era.<sup>39</sup> When Belzer and associates were able to eliminate the hemoglobin and hyperbaric chamber components for kidney preservation,<sup>40</sup> their asanguinous perfusion technique for cadaveric renal grafts became a worldwide standard. However, efforts were unsuccessful to use continuous asanguinous perfusion for livers.<sup>41</sup>



**FIG 11.**

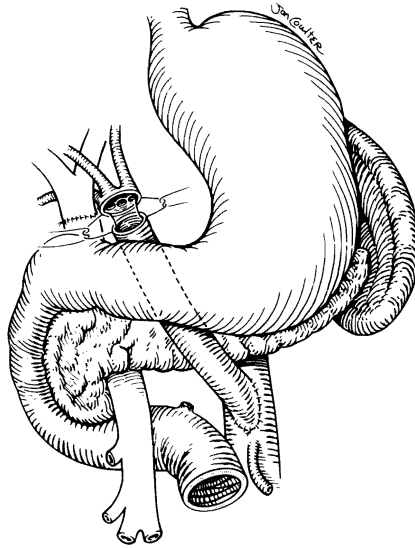
By 1979, all of the demonstrated grafts had been used clinically. The use of vascular grafts has been life saving, and liver transplantation should never be attempted without an emergency assortment of these grafts. (Redrawn from Starzl TE, Halgrimson CG, Koep LJ, et al: *Surg Gynecol Obstet* 1979; 149:76–77.)

### ***Slush Techniques***

The alternative strategy for the subsequent preservation of kidneys, livers, and other organs has been the instillation of special solutions (Table 1) such as those described by Collins and co-workers<sup>42</sup> or the plasma-like Schalm solution.<sup>43</sup> The original Collins solution or modifications of it have been used for almost 20 years for the so-called slush techniques of kidney preservation in which the organ is packed in an ice chest at 4°C after its infusion. The experimental work of Benichou and colleagues<sup>36</sup> and Wall and associates<sup>44</sup> with the Collins and Schalm solutions opened up the possibility in 1976 of clinical sharing of livers between cities but within narrow time limitations. The outer limit of safety for human livers was generally set at 8 hours in spite of the fact that dog livers could be maintained for much longer than this with the Collins and Schalm solutions.

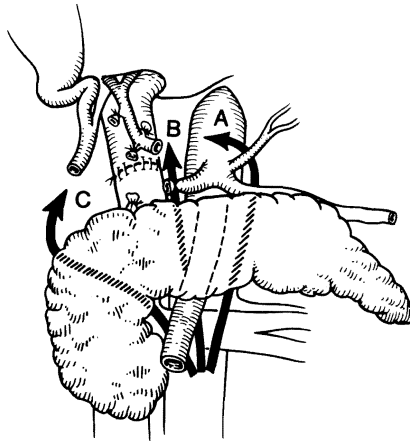
### ***The UW Solution for Slush Preservation***

The development of the UW solution has been the first major development in liver preservation since that time.<sup>45</sup> The UW solution is a generic advance that also is applicable to the preservation of the



**FIG 12.**

An antepancreatic route for a vascular graft placed onto the infrarenal abdominal aorta. The graft is brought either to the right or left of the middle colic vessels, anterior to the pancreas, and beneath the pylorus. (From Tzakis AG, Todo S, Starzl TE: *Transplant Int* 1989; 2:121. Used by permission.)



**FIG 13.**

Posterior routes by which the arterial grafts shown in Figure 11 can be brought from their aortic origin to the liver hilum behind the pancreas. The tunnels are created by blunt finger dissection. Route A is rarely used because it is potentially dangerous. (From Todo S, Makowka L, Tzakis AG, et al: *Transplant Proc* 1987; 19:2406–2411. Used by permission.)

**TABLE 1.**

Characteristics and Constituents of Test Solutions\*†

|   | EC   | LR  | UW    | UM1  | UM2  | UM3     |
|---|------|-----|-------|------|------|---------|
| <b>Anions</b>                                 |      |     |       |      |      |         |
| Bicarbonate (mM/L)                            | 10   | —   | —     | —    | —    | —       |
| Chloride (mM/L)                               | 15   | 109 | —     | —    | 20   | 5       |
| Lactate (mM/L)                                | —    | 28  | —     | —    | —    | —       |
| Phosphate (mM/L)                              | 57.5 | —   | 25    | —    | —    | 0.72    |
| Lactobionate (mM/L)                           | —    | —   | 100   | —    | —    | 90      |
| <b>Cations</b>                                |      |     |       |      |      |         |
| Calcium (mM/L)                                | —    | 1.5 | —     | —    | —    | 0.6–1.4 |
| Sodium (mM/L)                                 | 10   | 130 | 30    | 146  | 146  | 1       |
| Potassium (mM/L)                              | 115  | 4   | 120   | 4.3  | 24.3 | 124     |
| Magnesium (mM/L)                              | —    | —   | 5     | —    | 4    | 7.8     |
| <b>Colloids and osmotically active agents</b> |      |     |       |      |      |         |
| Hydroxy ethyl starch (gm/L)                   | —    | —   | 50    | —    | —    | —       |
| Proteins (gm/L)                               | —    | —   | —     | 65.2 | 90   | 65      |
| Mannitol (gm/L)                               | —    | —   | —     | —    | 3.75 | —       |
| Raffinose (gm/L)                              | —    | —   | 17.8  | —    | —    | 48      |
| Glucose (gm/L)                                | 194  | —   | —     | —    | —    | 99      |
| <b>Others</b>                                 |      |     |       |      |      |         |
| Adenosine (gm/L)                              | —    | —   | 1.34  | —    | —    | —       |
| Glutathione (gm/L)                            | —    | —   | 0.922 | —    | —    | —       |
| Insulin (units)                               | —    | —   | 100   | —    | —    | —       |
| Allopurinol (gm/L)                            | —    | —   | 0.136 | —    | —    | —       |
| <b>Antibiotics and steroids</b>               |      |     |       |      |      |         |
| Ampicillin (mg)                               | —    | —   | —     | —    | 250  | 50      |
| Sulfamethoxazole (mg)                         | —    | —   | 40    | —    | —    | —       |
| Trimethoprim (mg)                             | —    | —   | 8     | —    | —    | —       |
| Dexamethasone (mg)                            | —    | —   | 8     | —    | —    | —       |
| Methyl prednisolone (mg)                      | —    | —   | —     | —    | 250  | 500     |
| Osmolality (mOsm/L)                           | 375  | 273 | 320   | 308  | —    | 301     |
| pH  | 7.4  | 6.3 | 7.4   | 7.4  | 7.4  | 7.4     |

\*From Todo S, Podesta L, Ueda Y, et al: *Clin Transplant* 1989; 3:253–259. Used by permission.

†EC = Euro-Collins; LR = Lactated Ringer's; UW = University of Wisconsin; UM1 = University of Minnesota I; UM2 = University of Minnesota II; UM3 = University of Minnesota III.

pancreas,<sup>46</sup> kidney,<sup>47, 48</sup> and heart,<sup>49</sup> possibly by mechanisms common to all organs (see later).<sup>50</sup> The superiority of the UW solution to any previous infusion solution for preservation of the liver has been established in clinical trials.<sup>51–54</sup> In our trials,<sup>52–54</sup> the livers infused with UW solution performed better even though they were preserved on the average for almost twice as long as livers preserved with Euro-Collins solutions. The livers in UW solution permitted a higher rate of graft survival, and they had a lower rate of primary nonfunction, hepatic artery thrombosis and retransplantation. They appeared to be safe for at least 1 day and possibly longer.



When the UW solution was undergoing its first clinical trials, it was in such short supply that only a final portal flush was possible. In adult donors, 1 L of the UW solution was given on the back table via the portal vein just before packing the liver in ice. By this time, the liver had been cooled in situ with lactated Ringer's or Collins' solution and excised. In addition to a shortage of UW solution, the use of lactated Ringer's or Collins' solution for preliminary cooling was insisted on almost invariably by the local procurement team as a condition for the collaborative effort with the renal transplant surgeon.

The positive results clinically that have been reported with the UW solution were obtained with this mixed use of fluids in which only the final flush was with UW solution. However, the best practice probably is to use only UW solution for all infusions from the outset, a practice that became feasible (as of 1 June 1989) with the commercial availability of UW solution. The high cost of the UW solution (\$400/L) is the principal disadvantage of this practice. Approximately 5 L of solution are required for a multiple organ procurement in adults, exclusive of the thoracic organs. Because it has been shown that the UW solution is superior to previously used solutions for the kidney<sup>47, 48</sup> as well as the liver, there can no longer be any objection to this use of UW solution.

Although the extension with the UW solution of acceptable cold ischemia from 6 or 8 hours out to 1 day does not seem like a large gain, the effect has been phenomenal. Until 18 months ago, logistic problems dominated the use of cadaveric livers that had to be removed, transported to their destination, and revascularized with an overriding sense of urgency. With the longer preservation time that has been made practical with the UW solution, countrywide and worldwide networks of organ sharing have been set up. The consequences in the future should be a reduction in organ wastage, a greater flexibility for use of grafts that can be trimmed to the appropriate size for pediatric recipients, and more efficient recipient travel and preparation. The use of slower propeller planes instead of expensive jet planes for recipient and organ transplantation already has become feasible.

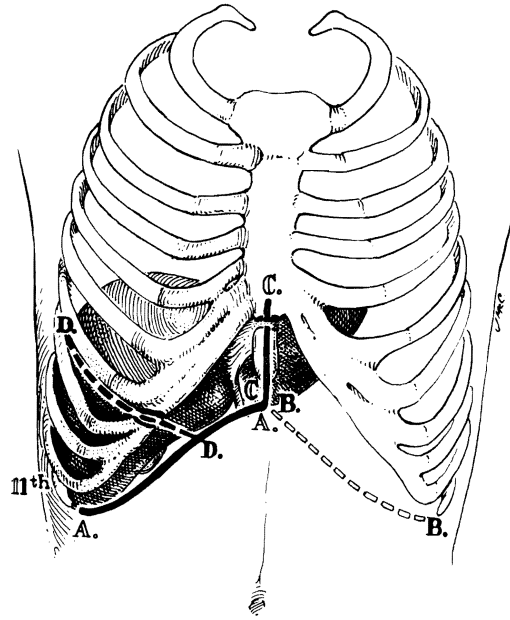
An explanation for the effectiveness of the UW solution has been provided by Belzer and Southard.<sup>55</sup> The UW solution contains more than 10 ingredients (see Table 1). Important components and their effects are (1) lactobionate and raffinose to prevent cell swelling, (2) hydroxyethyl starch to support colloidal pressure, (3) allopurinol and glutathione to inhibit oxygen free-radical generation, and (4) adenosine to enhance adenosine triphosphate (ATP) synthesis after reperfusion. In studies performed in our laboratory, the difference in the performance of different solutions may provide sketchy insight into the relative importance of some constituents.<sup>56</sup> These studies

have shown that an intracellular-like electrolyte solution (Collins) or a hyperosmolar colloid solution such as those developed at the University of Minnesota (see Table 1) do not allow long-term cold storage of the liver, even though these can be useful for the pancreas.<sup>57, 58</sup> Lactobionate and raffinose, two sugars that prevent imbibition of water by cells, have seemed to be the essential ingredients without which the effectiveness of UW solution is lost.<sup>56</sup> Others have come to the same conclusion.<sup>59, 60</sup>

Much remains to be learned about liver preservation, the effects of ischemia on hepatic function and the hepatic microvasculature, and the role of these factors in the early and late postoperative course of recipients. We will return to these subjects further on. In the meanwhile, a discussion of slush preservation would be incomplete without mentioning the potential dangers of the preservation solutions. For example, the bolus of potassium washed out during the reperfusion of a liver containing Collins' or UW fluid has caused a number of cardiac arrests. It also should be noted that other ingredients than potassium in present-day preservation solutions may impose a risk. Prien and associates have shown that bradycardia or even more serious arrhythmias are caused in recipients of kidneys preserved with UW solution if these organs are not washed out first.<sup>61</sup> They believe that the offending agent is adenosine, which is known to be arrhythmogenic. Aside from this consideration, and the elimination of potassium, the preservation fluid should be washed out of liver grafts before they are placed into the recipient circulation to eliminate air bubbles entrapped in the graft.<sup>62</sup> Moen and co-workers have shown that sodium can be substituted for potassium in the UW solution.<sup>60</sup> This change will make safer the reperfusion of liver grafts by eliminating the potential bolus of potassium at the time of reperfusion.

#### *RECIPIENT OPERATION*

The component parts of the recipient operation are so dissimilar that a single surgeon operating from skin to skin may find it difficult to adjust to the changing pace. Removal of the diseased liver can be one of the most bloody and stressful experiences in a surgeon's life. Yet, the subsequent performance of the vascular anastomoses can be among the most delicate and sophisticated, especially in very small children. Obtaining perfect hemostasis subsequently is often a tedious third phase that, if not accomplished, will ruin all that has gone before. At the end, success depends on adequate biliary tract reconstruction. In some centers, various parts of the procedure are being done by independent and fresh teams. However, the total re-



**FIG 14.**

The usual incision used for the recipient of an orthotopic liver graft. A right subcostal incision is always made, usually with an upper midline extension, and often with a left subcostal extension. Removal of the xyphoid process gives extra exposure of the suprahepatic vena cava. (From Starzl TE, Bell RH, Beart RW, et al: *Surg Gynecol Obstet* 1975; 141:429–437. Used by permission.)

sponsibility still rests with a single surgeon who must understand each part of the operation.

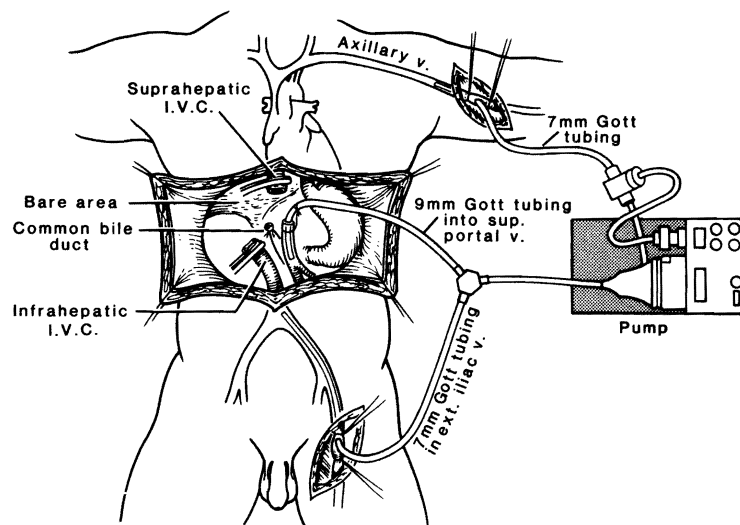
A right subcostal incision is almost always used for the recipient operation (Fig 14), but its exact location is dictated by previous right upper quadrant incisions and by the size and configuration of the liver. An upper midline extension has been particularly valuable. If the upper midline extension is made, the xiphoid process usually is excised since better access to the hepatic veins and suprahepatic vena cava can be obtained. In the majority of cases, the patients end up with a bilateral subcostal incision, with a superior midline T extension (see Fig 14). Thoracic extensions are almost never needed.

Once the abdomen is entered, an effort is made to find a plane of dissection just outside of the liver capsule if there are major adhesions. Movement away from this plane invites disruption of varices that may be large enough to cause unpleasant or even lethal hemorrhage during the preliminary dissection.

### Veno-Venous Bypasses

During recipient hepatectomy and performance of the vascular anastomoses of the homograft, the portal vein and inferior vena cava are crossclamped (Fig 15). The choice of the dog in 1958 as the species to develop the operation focused attention immediately on the need to decompress the acutely obstructed venous beds. The normal dog cannot tolerate venous hypertension of the splanchnic capillary bed for more than 15 or 20 minutes without the development of hemorrhagic necrosis of the intestinal mucosa. Passive veno-venous bypasses from the stagnant venous pools to the upper part of the dog's body can circumvent these lethal complications without the need for heparinization.<sup>6,8</sup>

Such passive bypasses were used for several patients in the first clinical trials<sup>63,64</sup>; however, either the bypasses clotted and did not function at all or, far worse, clots were released from the bypass tubing and passed to the lung, causing lethal pulmonary emboli.<sup>64</sup> In addition, it quickly was appreciated that the human can tolerate obstruction of the inferior vena cava and portal vein better than the dog, that other species, including the pig, were more like humans in this respect, and that even in the dog venous crossclamping could be made safer by the expedient of bile duct ligation several weeks in advance.<sup>65</sup> The logical conclusion from this last observation was that



**FIG 15.**

Pump-driven veno-venous bypass, which allows decompression of the splanchnic and systemic venous beds without the need for heparinization. (Redrawn from Griffith BP, Shaw BW Jr, Hardesty RL, et al: *Surg Gynecol Obstet* 1985; 160:270–272.)

the stimulation of venous collaterals by liver disease diminished the magnitude of venous hypertension caused by acute venous obstruction. Efforts to decompress the obstructed venous beds were abandoned by 1964 and were not resumed for almost 20 years.

So persuasive were the arguments against using veno-venous bypasses that liver transplantation was repetitively performed in humans under conditions that limited its usefulness, increased its perioperative risk, and made training of the next generation of hepatic surgeons difficult. Liver transplantation was widely viewed as being too dangerous and difficult to be generally applicable. The mistake had been made of believing that a fundamental principle of surgical physiology worked out in animals, namely that veno-venous bypass was essential for effective liver transplantation, was not truly relevant in humans.

It was possible to carry out liver transplantation successfully without veno-venous bypasses,<sup>66, 67</sup> but the operation could be performed only by highly experienced surgeons and frequently with such a sense of urgency that training of new teams in any numbers was not possible. All too often, a virtuoso performance was required, and even when the anhepatic period was kept to a minimum, major declines in cardiac output and variable hypotension were common.<sup>68</sup> The fact that recovery usually occurred in the hands of skilled teams created a false impression about the expendability of the bypass. Usually there was gross swelling of the intestine during the period of occlusion. Subsequently, many patients suffered from third space sequestration and postoperative renal failure. The extent to which these complex physiologic events contributed to the high perioperative morbidity of the 1960s and 1970s was not fully appreciated until later.<sup>67, 69</sup>

How this deficiency in technique was rectified cannot be traced easily from the articles describing the work. The stimulus for reassessment was a persistent 5% to 10% intraoperative mortality that was due almost entirely to poor patient tolerance of the venous occlusions during the anhepatic phase. However, nothing decisive was done to rectify the situation until a tragedy occurred in Pittsburgh in May 1982 that utterly demoralized the transplant team. A popular male hemophiliac teenager with chronic active hepatitis died on the operating table from the combination of bleeding, third space fluid sequestration, and cardiovascular instability that was then common during hepatectomy and the sewing in of the new liver.

The program was closed for more than 1 month until June 15, 1982 when cardiac surgeon Dr. Henry T. Bahnson, Chairman of the Department of Surgery at the University of Pittsburgh, was requested to set up a pump-driven bypass for the next case. Bahnson grasped the essence of the problem instinctively, and he agreed immediately. That night, a liver replacement was carried out under veno-venous

bypass in a 6-year-old child with biliary atresia. The bypass was performed under 3 mg of heparin/kg with a roller pump and other conventional equipment used for open-heart surgery. This technique of a pump-driven bypass had been described in dogs 10 years earlier by Cutropia and associates,<sup>70</sup> but their article was unknown to us at the time. There was little trouble in reversing the heparin effect afterward. Those who were there that night were ecstatic about the ease and nonstressful nature of the transplantation under bypass conditions.

The ways in which liver transplantation was facilitated by venovenous bypass were verified in a number of other cases.<sup>67</sup> By July 1982, abstracts describing the technique were submitted under the senior authorship of Bahnson to the Southern Surgical Association and to the American Association for the Study of Liver Diseases. Both were rejected. In the meanwhile, problems with reversal of the heparin effect had been encountered in several of the adult recipients. Venovenous bypass under systemic heparinization had worked well in those patients with relatively "simple" diseases such as primary biliary cirrhosis and in recipients who had not had previous abdominal operations. The same was not true in patients with difficult pathology, exceptionally advanced disease, and especially in those who had undergone multiple procedures previously. Here, the bleeding from the raw surfaces was so great and the heparin effect reversed with such difficulty that the value of bypass technique was vitiated. In fact, two patients with venovenous bypass under heparin died of hemorrhage when clotting could not be restored.

Two of Bahnson's young associates, Drs. Bartley Griffith and Robert Hardesty, had avoided systemic heparin in patients with pulmonary insufficiency who had been treated with pump-driven extracorporeal membrane oxygenators. Griffith and Hardesty recently had purchased an atraumatic centrifugal pump that they thought would permit the pumping of venous blood without anticoagulation. Work on the nonheparin bypass began in dogs in the laboratory on September 30, 1982. The project was assigned to Dr. Scot Denmark, a resident who was in his "lab year." Griffith and Denmark provided the bypass capability. The liver transplantations were performed by members of the transplantation service, including the second-year transplantation fellow Dr. Byers Shaw, Jr. By the end of 1982, most of the work that was reported by Denmark at the Surgical Forum of the American College of Surgeons in October 1983 already had been completed.<sup>71</sup> However, clinical trials of the nonheparin bypass were not started, in part because it was difficult to predict which patients really needed it. In addition, there still was uneasiness about the possibility of clot formation in bypass tubing and consequent pulmonary emboli.

During the Christmas season of 1982 and in January 1983, three

more deaths occurred on the operating table in much the same way as with the earlier hemophiliac patient. As a consequence, a policy decision was made at the end of January 1983 that veno-venous bypasses must be used for all adult recipients of liver transplants from that time onward (see Fig 15). It became obvious almost immediately that liver transplantation had become a far more reasonable procedure than in the past.<sup>69, 72</sup> Kam and associates have shown subsequently that these techniques are easy to use and safe in many pediatric recipients, particularly those weighing more than 15 kg.<sup>10</sup>

Not all liver transplant surgeons believe that veno-venous bypasses are of overriding importance.<sup>73-77</sup> Calne and co-workers have described a venoarterial bypass, sometimes with an intervening oxygenator, that is used only when venous cross clamping causes cardiodynamic instability.<sup>73</sup> They contend that strain on the heart is relieved thereby. Even today, most infants and small children undergo liver transplantation without veno-venous bypass, and some surgeons routinely omit it for their adult recipients.<sup>74-77</sup> Nevertheless, veno-venous bypass converted liver transplantation to a procedure that can be carried out by many well-trained general or vascular surgeons. The consequence was that effective teams could be developed quickly, blanketing the United States and Europe almost overnight with a network of competent liver transplant services.

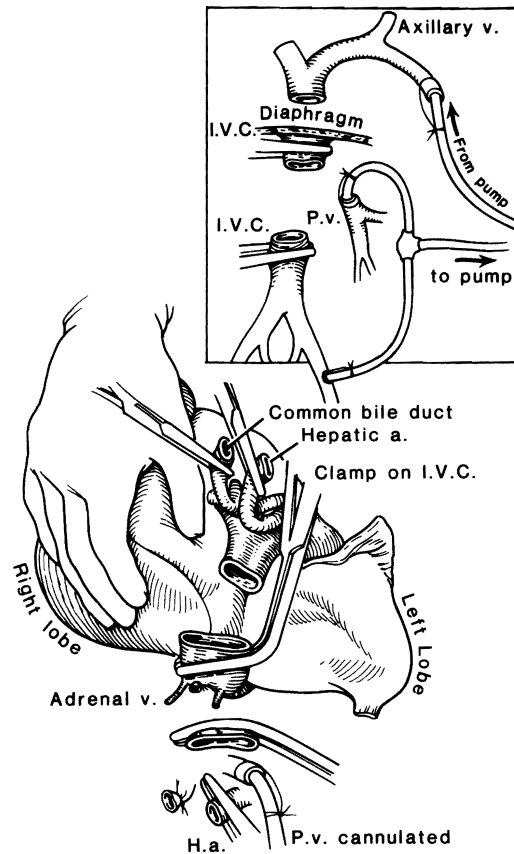
#### *Recipient Hepatectomy*

There is no single best way to remove a diseased native liver. In each case, an ad hoc decision is required on the best technical approach that the abnormal anatomy will permit. In some patients, efforts to mobilize the liver from the hepatic fossa can cause lethal hemorrhage unless the hepatic arterial and portal venous blood supply are ligated first. In the other recipients, it may even be impossible because of scarring from previous operations or because of the massive formation of varices to dissect individually the structures of the portal triad.

Finally, the method of hepatectomy, as well as the conduct of the rest of the operation, are determined largely by whether or not veno-venous bypasses are going to be used. If the bypass is omitted, it is important to limit the venous occlusion period as much as possible, hopefully to the time required for performance of the two vena caval and the portal anastomoses. Otherwise, damage to the splanchnic and systemic capillary beds may be excessive, with grossly obvious petechial hemorrhages and edema in the intestines and elsewhere. With occlusion of both the vena cava and the portal vein, hemorrhage from the thin-walled varices and from all other raw surfaces of the operative wound is predictably amplified. The bleeding often cannot be controlled by any mechanical means until decompression is accomplished by opening of the vena caval and portal venous

anastomoses of the new liver. Thus, if veno-venous bypasses are to be omitted, as much preliminary dissection as possible is desirable so that the occlusion period can be made as short as possible.

In contrast, the extent of preliminary dissection can be greatly decreased if a veno-venous bypass is to be used. The individual structures of the hilum usually are skeletonized, but no other areas need be invaded. When the bypass is ready for implementation, the hepatic artery and the common duct are ligated. The portal vein cannula for the veno-venous bypass is inserted, as is a femoral cannula, allowing both the splanchnic and systemic systems to be brought into the veno-venous circuit (see Fig 15). Entry into the superior vena



**FIG 16.**

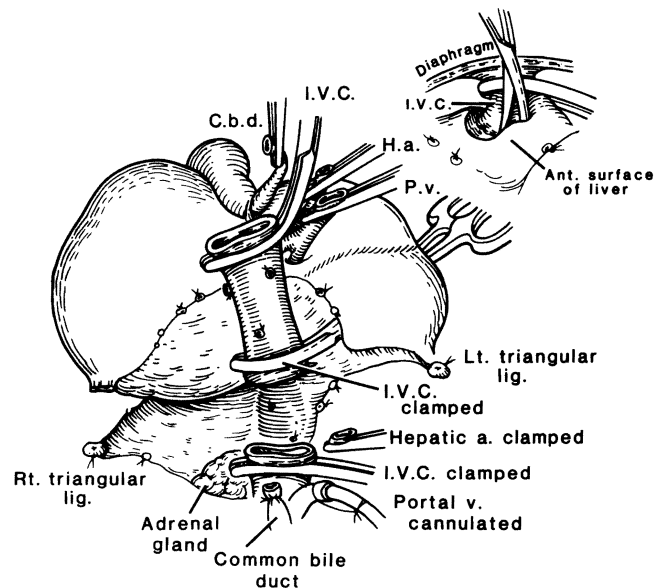
Technique for removal of the liver from below upward. This is a particularly attractive approach if a veno-venous bypass is used as shown. (Redrawn from Starzl TE, Porter KA, Putnam CW, et al: *Surg Gynecol Obstet* 1976; 142:487-505.)



caval system usually is via the axillary vein (Fig 16). In adults, 1 to 6 L of blood per minute are bypassed. Simultaneous obstruction of the portal vein and inferior vena cava should cause little change in blood pressure or other measures of cardiovascular function.

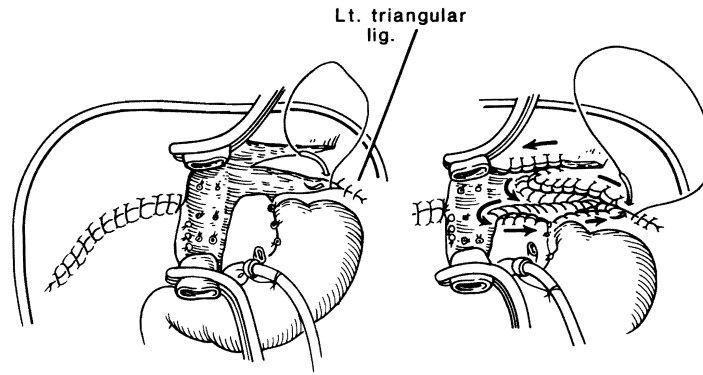
With the hemodynamic stability afforded by the veno-venous bypass, it is possible to systematically dissect all other structures that are holding the now-devascularized liver, including the infrahepatic vena cava. The triangular ligaments and the leaves of peritoneal reflection that make up the coronary ligament are cut if these have not been incised already (Fig 17). The bare areas are entered on both the right and left sides. After these maneuvers have been carried out, the right hepatic lobe can be retracted into the wound. If it has not been possible to encircle the inferior vena cava earlier, this can be done now just below or above the liver, and eventually at both locations. The liver can then be shelled out on the stalk defined by the vena caval connection (see Fig 17), and the vena caval cuff for eventual anastomosis can be developed (see Fig 17, inset).

Once the liver has been removed, it is possible using veno-venous bypass time to close most of the raw surfaces that were created dur-



**FIG 17.**

Completion of removal of the liver from below upward, leaving the liver attached only by a stalk of vena cava at the diaphragm. (Redrawn from Starzl TE, Porter KA, Putnam, CW, et al: *Surg Gynecol Obstet* 1976; 142:487–505.)



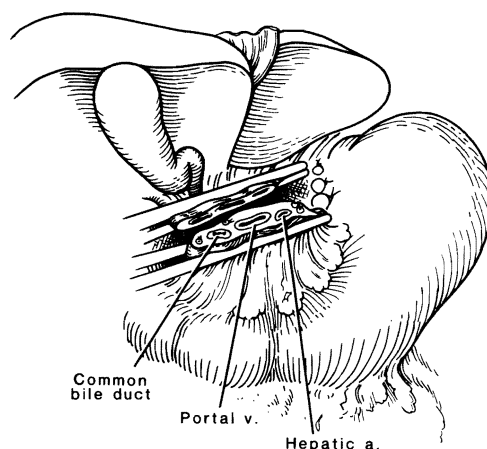
**FIG 18.**

Closure and hemostasis of the bare area after peeling out the liver. Under conditions of veno-venous bypass, there is plenty of time to do this so that the wound is dry when the anastomoses to the new liver are carried out. (Redrawn from Starzl TE, Iwatsuki S, Shaw BW Jr, et al: *Transplant Proc* 1985; 17:107–119.)

ing the hepatectomy (Fig 18). Closure is usually done with a continuous polypropylene suture, beginning at the tip of the right triangular ligament and continuing centrally in rows that eventually are connected.<sup>78</sup> The superior leaf of the coronary ligament can be the starting point, with continuation into the bare area itself and eventually to the inferior portion of the coronary ligament (see Fig 18). When these continuous suture lines are eventually incorporated into a single suture line, all of the right bare area may be eliminated if desired. The same principle is followed in dealing with the left triangular and falciform ligaments as well as other bare areas (see Fig 18).

The foregoing well-ordered strategy of hepatectomy is not always possible, particularly in patients who have undergone previous operations in the upper abdomen. In some cases, the only way to get the liver out is by placing clamps across the entire hilum. The lumens of the individual hilar structures can then be seen after transecting between the mass clamps, and these individual structures can be dissected downward toward the clamp (Fig 19).

Another drastic variation that may be especially helpful in children in whom the infrahepatic vena cava is inaccessible is to encircle and transect the vena cava above the liver. When this is done, one or two fingers are thrust downward into the retrohepatic vena cava to prevent massive hemorrhage (Fig 20). Then the liver can be peeled down from above.



**FIG 19.**

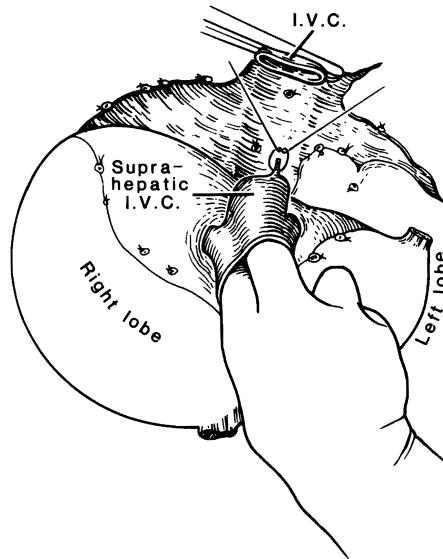
Crossclamping of portal triad, which is sometimes necessary when preliminary dissection of the individual structures is too difficult. Once the triad has been clamped, the triad structures are seen head on and are dissected downward. A portal venous cannula for veno-venous bypass can then be inserted. (Redrawn from Starzl TE: *Experience in Hepatic Transplantation*. Philadelphia, WB Saunders Co, 1969.)

#### ***Preservation of the Vena Cava and the Piggyback Technique***

An integral part of the standard orthotopic liver transplantation is removal of the inferior vena cava from above the renal veins to the diaphragm. Complete excision of this retrohepatic vena cava is not necessary, and Stieber and co-workers have pointed out that it may not be desirable.<sup>79</sup> They recommend leaving that portion of vena cava into which the right adrenal vein drains and then over-sewing it.

In another modification, the full length of the recipient inferior vena cava is preserved, and the new liver is placed "piggyback" onto its anterior surface. A particularly appealing feature of the piggyback operation in children for whom veno-venous bypass might not be feasible is that vena caval occlusion can be avoided during the hepatectomy and sewing in of the homograft. The piggyback operation has been used for a number of years. In some of our first patients, this operation was employed,<sup>80</sup> and one of Calne's first five recipients had a piggyback operation.<sup>81</sup> However, the formal description and widespread use of the piggyback operation has been recent.<sup>82</sup> At the present time, about one fifth of our recipients are having the piggyback modification.

The essence of this operation is shown in Figure 21. By rotating



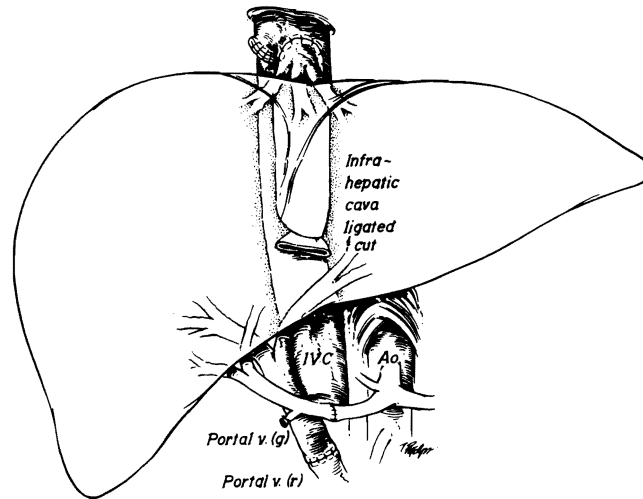
**FIG 20.**

Removal of the liver from above downward, preventing hemorrhage with a finger or fingers thrust down the lumen of the transected suprahepatic vena cava. The maneuver is indicated if it is difficult or impossible to safely encircle the inferior vena cava below the liver. This technique is almost specific for certain cases of biliary atresia with extensive subhepatic scarring in which the small size of these livers makes it possible to completely occlude the vena cava with a single finger. (Redrawn from Starzl TE, Iwatsuki S, Shaw BW Jr, et al: *Transplant Proc* 1985; 17:107-119.)

the liver out of the wound, either to the right or to the left, one can dissect the individual hepatic veins, ligate them, and divide them (Fig 22). The major hepatic veins are crossclamped and eventually used to fashion an orifice for the outflow anastomosis of the homograft (Fig 23). The right, middle, and left hepatic veins or, more commonly, the middle and left are joined by dividing the intervening septum.

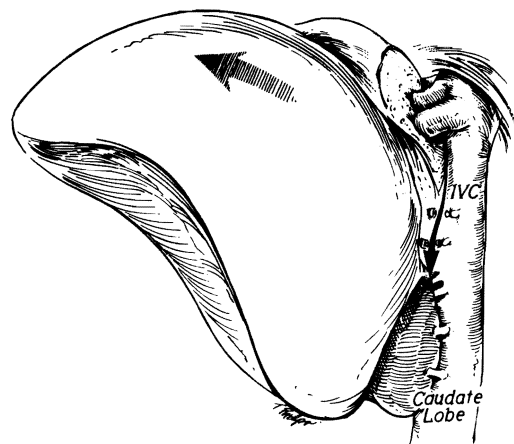
If difficulty is encountered in dissecting the hepatic veins from an exterior approach, an alternative technique is to split the liver like a book. A tributary-free plane is identified at the upper portion of the liver, and with gentle blunt dissection, the finger is burrowed down the anterior surface of the vena cava (Fig 24). The liver is then divided with a knife from its anterior surface down to the finger using a knife (Fig 25).

The exact technique of the outflow anastomosis depends on which hepatic veins have been selected for this purpose. The lower end of the vena cava of the homograft is ligated or sutured (see Fig 21).



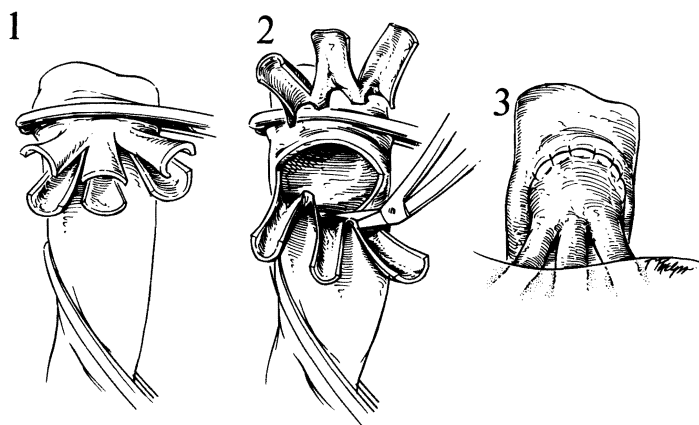
**FIG 21.**

Transplantation of a liver piggyback onto an inferior vena cava, which is preserved through its length. Note that the suprahepatic vena cava of the homograft is anastomosed to the anterior wall of the recipient vena cava. The retrohepatic vena cava of the homograft is sutured or ligated, leaving a blind sac into which empty numerous hepatic veins. (From Tzakis A, Todo S, Starzl TE: *Ann Surg* 1989; 210:649–652. Used by permission.)



**FIG 22.**

Steps in preparation of the recipient vena cava for the piggyback operation. All of the small hepatic veins entering the retrohepatic vena cava are ligated and divided, and the large principal tributaries (right, middle, and left hepatic veins) are dissected free. (From Tzakis A, Todo S, Starzl TE: *Ann Surg* 1989; 210:649–652. Used by permission.)



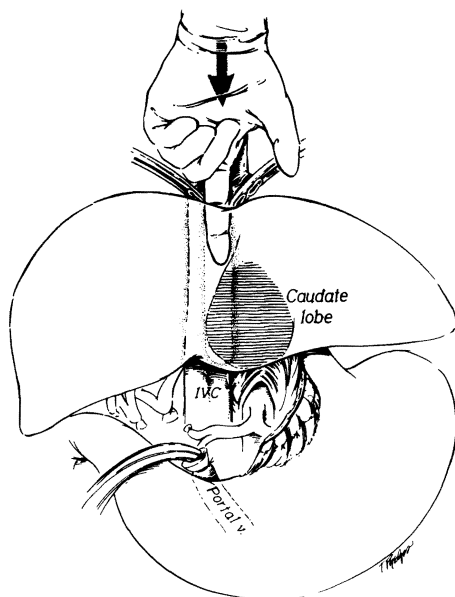
**FIG 23.**

Formation of the site for anastomosis. The three main hepatic veins can be connected as shown, or other combinations can be used, of which the most common is a left and middle hepatic cloaca. (From Tzakis A, Todo S, Starzl TE: *Ann Surg* 1989; 210:649–652. Used by permission.)

The applicability of the piggyback operation depends on finding favorable anatomic conditions as the recipient hepatectomy proceeds. If the liver is very cirrhotic, small, and firmly adherent to its retrohepatic vena cava, it is foolish to persist in efforts to save the vena cava. When it is easy to perform, there is little that can be said in criticism of this variant technique, which is being used in about 75 cases per year in our Pittsburgh program.<sup>82</sup>

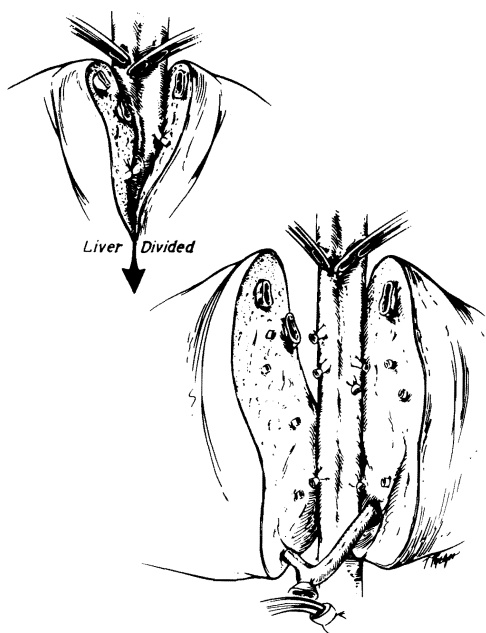
#### **Graft Revascularization**

In most cases, anastomoses of the vena cava above and below the liver are performed first. While the lower vena cava anastomosis is being constructed, the liver is flushed with lactated Ringer's solution to remove entrapped air from its major veins and to rid the graft of the highly concentrated potassium that is contained in the preservation fluid (Fig 26). Failure to observe these precautions can result in air embolus or in cardiac arrest from hyperkalemia.<sup>62</sup> The portal venous anastomosis usually is done next, and the liver is revascularized with a portal blood supply. A very aggressive effort then is made to find major bleeders and to control these before proceeding with rearterialization. As already mentioned under donor hepatectomy, many options have been described for dealing with anomalies or other unusual anatomic features of the donor or recipient arteries. The objective in all is to obtain as large a caliber recipient vessel as possible, consistent with the size of the donor artery. This usually



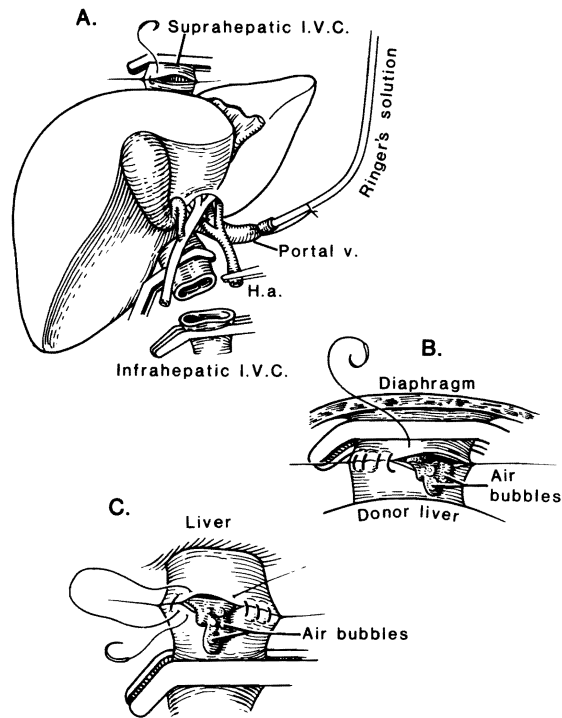
**FIG 24.**

Maneuver that facilitates cleaning the dissection of the retrohepatic vena cava and removing the liver. A vascular plane exists on the anterior surface of the retrohepatic vena cava, which is developed with a gently inserted finger. (From Tzakis A, Todo S, Starzl TE: *Ann Surg* 1989; 210:649–652. Used by permission.)



**FIG 25.**

After the plane is developed as shown in Figure 24, the liver is boldly transected, bringing into view the vena cava, and the right and left fragments are removed as quickly as possible. (From Tzakis A, Todo S, Starzl TE: *Ann Surg* 1989; 210:649–652. Used by permission.)



**FIG 26.**

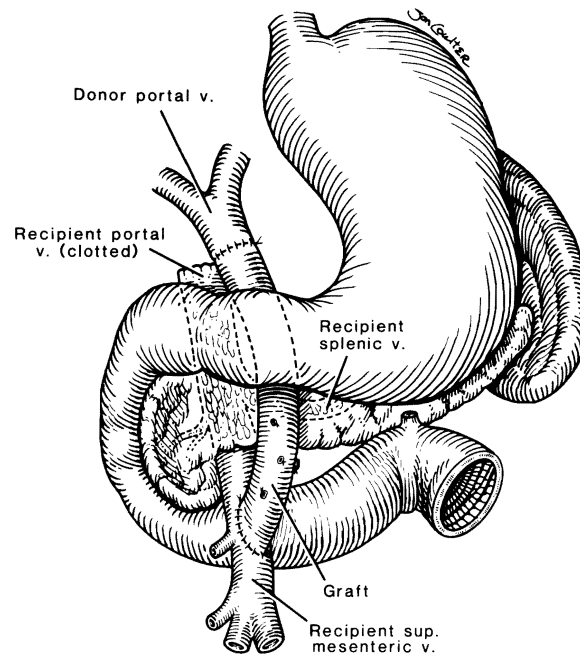
Technique of washing out the homograft, which is performed with both the piggyback and standard operations. The organ is washed with a solution of low potassium concentration (**A**) to avoid the infusion of a bolus of potassium from the preservation fluid during revascularization. In addition, it is important to wash out air that may be trapped in the large hepatic veins (**B** and **C**). Failure to eliminate these bubbles could lead to air embolism. (Redrawn from Starzl TE, Schneck SA, Mazzoni G, et al: *Ann Surg* 1978; 187:236–240.)

requires making the anastomosis proximal to the gastroduodenal artery in recipients with normal arterial anatomy and often proximal to the splenic and left gastric arteries in those recipients with anomalies.

Vein grafts of the portal vein can be inserted above the pancreas, usually at the confluence of the splenic and superior mesenteric vein (see Fig 11). If a portal vein thrombosis extends too far distally to allow insertion of a vein graft superior to the pancreas, a jump graft can be placed on the anterior surface of the superior mesenteric vein below the transverse mesocolon. The graft is brought anterior to the pancreas and beneath the pylorus (Fig 27).

In restoration of the portal venous and hepatic arterial circula-

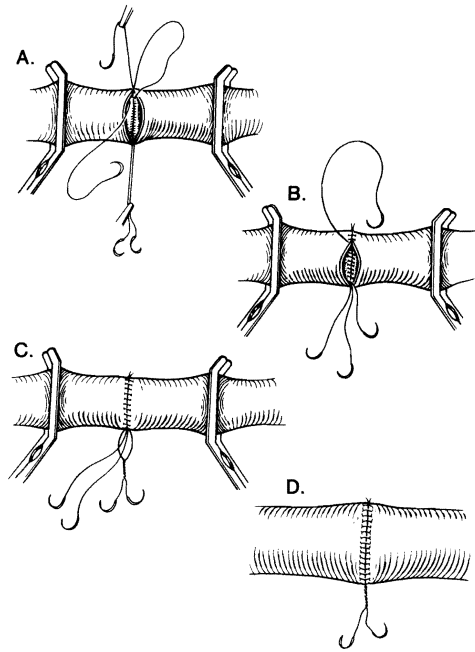




**FIG 27.**

The use of an antepancreatic portal vein graft from the superior mesenteric vein through the same pathway as shown for an arterial graft in Figure 12. The use of these grafts has eliminated portal vein thrombosis as a contraindication to transplantation, providing a good superior mesenteric vein is still open. (From Tzakis A, Todo S, Stieber A: *Transplantation* 1989; 48:530–531. Used by permission.)

tions, performance of a poor anastomosis with subsequent thrombosis usually will cause death or necessitate retransplantation. We have described special techniques to prevent flawed anastomoses, particularly in children who have small vessels.<sup>83</sup> These special techniques were designed to prevent anastomotic strictures.<sup>83</sup> The anastomoses are done in the usual way with a continuous polypropylene suture (Figs 28,A and B), but a so-called growth factor is left by tying the sutures at a considerable distance from the vessel wall (Fig 28,C). After flow is restored through the hepatic artery or portal vein, the excessive suture recedes back into the vessels and distributes itself throughout the circumference of the suture line (Fig 28,D). If an additional suture is placed at the point where the two ends of the continuous suture line meet, thus preventing distraction of the lips at this point, the amount of hemorrhage at the time of flow restoration is surprisingly small. Suture materials other than polypropylene are



**FIG 28.**

Method of avoiding strictures of small vascular anastomoses. See text for explanation. (Redrawn from Starzl TE, Iwatsuki S, Shaw BW Jr: *Surg Gynecol Obstet* 1984; 159:164–165. Used by permission.)

not satisfactory for this technique. The polypropylene is so slippery that it is not caught by the adventitia and can easily work itself back through the entire circumference of the suture line.

### ***Biliary Reconstruction***

An acceptable technique of biliary tract reconstruction if the anatomic conditions permit is end-to-end anastomosis of the donor and recipient common ducts over a T-tube stent (see Fig 1).<sup>67</sup> Variants of this principle include a side-to-side choledochocholedochostomy after closure or ligation of the donor and recipient duct ends.<sup>84</sup> Alternatively, the homograft common duct can be anastomosed to a defunctionalized (Roux) limb of jejunum (see Fig 1) with equally good results.<sup>67, 85, 86</sup> Whichever method is used, there has been a 10% to 15% incidence of late bile duct obstruction that required correction by interventional radiologic techniques, secondary duct reconstruction, or in occasional cases with retransplantation.<sup>85–89</sup>

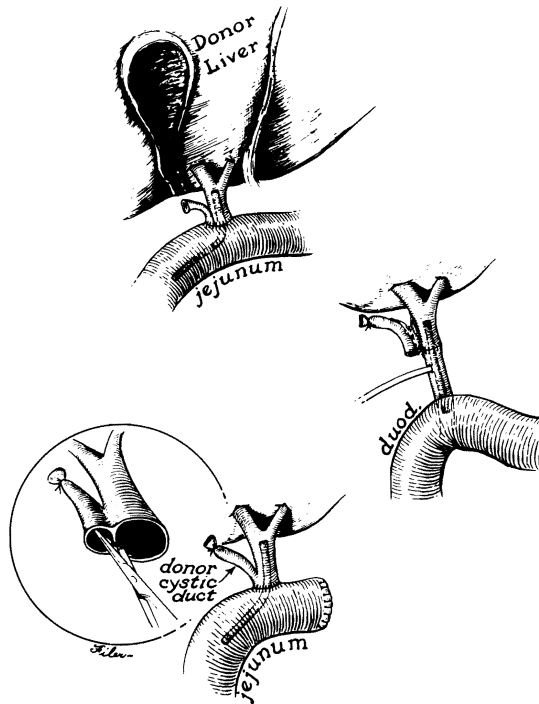


**FIG 29.**

Typical extrinsic mass effect on homograft common hepatic duct, which is caused by obstructing a cystic duct at both ends. This surgical complication results in obstructive jaundice and requires secondary excision of the resulting mucocele. (From Koneru B, Zajko AB, Sher L, et al: *Surg Gynecol Obstet* 1989; 168:394–396. Used by permission.)

One kind of biliary obstruction that is highly avoidable is caused by leaving an obstructed segment of the cystic duct with the graft. Usually, this occurs when the cystic duct enters the common duct at an anomalously low level, creating a double lumen at the site of duct transection. If the distal and proximal ends are occluded, a mucocele can form in the obstructed segment and lead to extrinsic compression (Fig 29).<sup>90</sup> The best way to avoid this is to completely resect the cystic duct at the time of transplantation. Alternative techniques are shown in Figure 30.

Rarely, there may be an indication to use a technique that incorporates a donor gallbladder conduit between the donor common duct and the recipient anastomotic site.<sup>91,92</sup> This method (Fig 31), which was described by Waddell and Grover<sup>91</sup> and by Calne,<sup>92</sup> has had a high incidence of late sludge and stone formation. In our experience, almost one half of the biliary tracts reconstructed with the Waddell-Calne technique eventually developed the characteristic ob-



**FIG 30.**

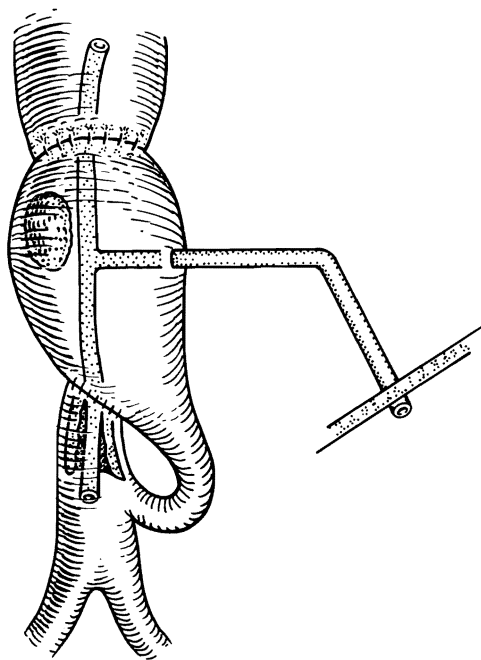
The best way to prevent mucocoele formation is to completely excise the cystic duct, but alternative techniques to prevent a blind cystic duct remnant are shown. (From Koneru B, Zajko AB, Sher L, et al: *Surg Gynecol Obstet* 1989; 168:394–396. Used by permission.)

struction shown in Figure 32.<sup>93</sup> It has been possible to rectify the situation by conversion to a choledochojejunostomy.

#### *Need for Hemostasis*

Complete hemostasis is mandatory before closing. The assumption that nature will take care of bleeding if effective liver function is provided by a homograft has proved to be a vain hope on many occasions. Often a coagulopathy will be present intraoperatively that can persist into the postoperative period.

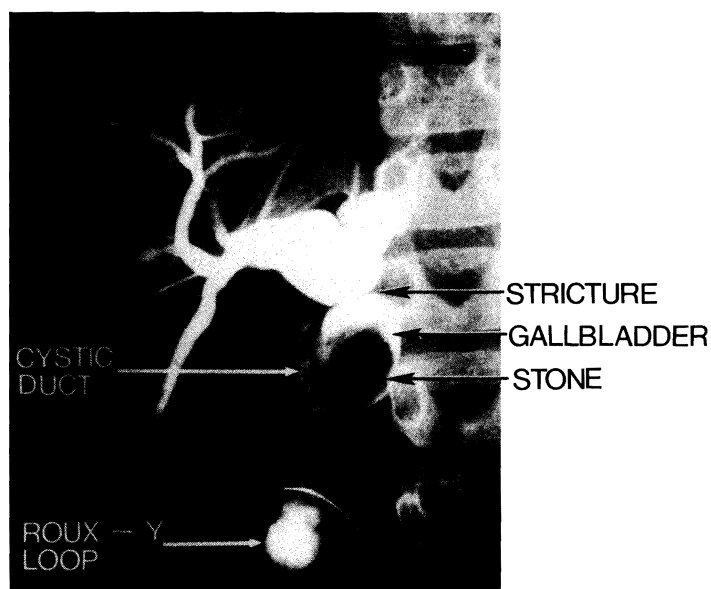
The presence of the coagulation expert Dr. Kurt von Kaulla at the University of Colorado in the 1960s was a key element in the development of the transplantation programs there. Von Kaulla and associates studied the renal<sup>94</sup> and hepatic<sup>95</sup> recipients and characterized the clotting defects in both classes of patients. In the first three liver recipients, they demonstrated clotting factor defects, showed the seriousness of fibrinolysis as well as how to treat this problem.<sup>63</sup> They



**FIG 31.**

The Waddell-Calne technique of gallbladder conduit biliary reconstruction. Note that the homograft common duct has alternative pathways of emptying, both through the gallbladder conduit. The choledochocystostomy is stented with a T tube brought out through the gallbladder. (From Half G, Todo S, Hall R, et al: *Transplantation* 1989; 48:537–539. Used by permission.)

recommended the thromboelastogram to follow the minute-to-minute clotting changes in the operating room in much the same way as is recommended and practiced currently. Other studies showing consumption of clotting factors, including platelets within the graft itself<sup>96, 97</sup> and the development in some patients of a hypercoagulable state postoperatively, completed the picture. Flute of Cambridge provided confirmatory data.<sup>98</sup> Ultimately, this kind of information was acted on systematically for therapeutic correction by the anesthesiologists at the University of Pittsburgh in the early 1980s under the direction of Drs. Jessica Lewis, Frank Bontempo, and Yoo Goo Kang.<sup>99–103</sup> Now cautious correction of coagulation defects is an integral part of liver transplantation, greatly diminishing the hemorrhages of nightmare proportions that were common. As already emphasized, the other factor that has ameliorated the intraoperative bleeding problems has been the systematic use of venovenous bypasses.



**FIG 32.**

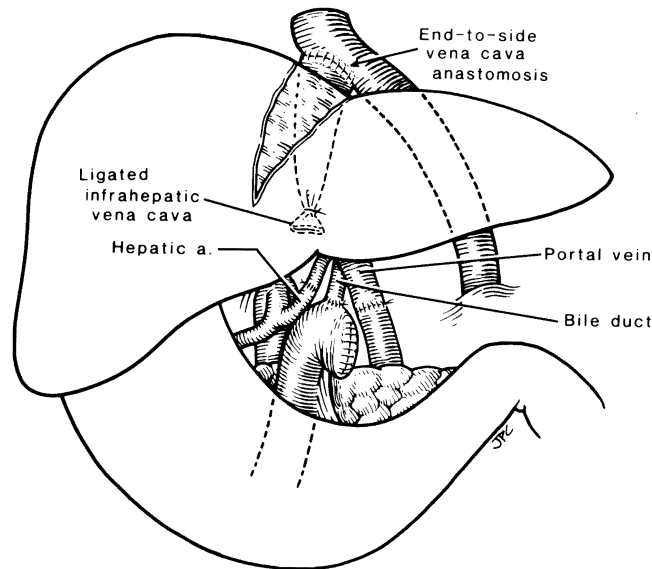
Typical complication with the Waddell-Calne reconstruction. (From Halff G, Todo S, Hall R, et al: *Transplantation* 1989; 48:537-539. Used by permission.)

Many hours of tedious and exhausting effort may be necessary to obtain perfect hemostasis, but these efforts are eventually rewarded with a dry wound. After hemostasis has been accomplished, closed sump drains are placed in two or three locations above and below the liver, and the wound is closed with nonabsorbable sutures.

#### ***Modifications of This Standard Procedure***

The piggyback operation, in which the graft is placed onto the anterior surface of the retained recipient inferior vena cava, was mentioned previously. The other structures are anastomosed in the usual way. The piggyback reconstruction gives an unusual degree of mobility to the liver and a greater freedom in tailoring vessel lengths. These may be important advantages if the donor liver is substantially smaller than the diseased native organ that was removed. The piggyback operation has also been especially helpful for four of our patients with situs inversus, of whom one has been reported (Fig 33). A patient with situs inversus also has had an orthotopic liver transplantation performed by Raynor and colleagues, with removal of the vena cava in the usual way.<sup>104</sup>

Size reduction techniques that permit the transplantation of part of a liver have been perfected in recent years in Paris,<sup>105, 106</sup> Hannover,<sup>107, 108</sup> Brussels,<sup>109</sup> and Chicago,<sup>110, 111</sup> allowing greater flexibil-



**FIG 33.**

Reconstruction after transplantation to a child with situs inversus. Note suprahepatic inferior vena cava of the graft was anastomosed to the anterolateral surface of the recipient inferior vena cava. The graft infrahepatic vena cava was ligated. (From Todo S, Hall R, Tzakis A, et al: *Clin Transplantation*, in press. Used by permission.)

ity in matching donor availability to recipient needs. Pediatric recipients have benefitted most from this development. The first known example of partial liver transplantation occurred on March 26, 1975 at the University of Colorado. The left lateral segment of an adult liver was transplanted into the orthotopic position in an infant with biliary atresia. Because of its historic interest, the case is described here.

The recipient, a 23-month-old boy weighing 8.2 kg, had a failed Kasai portoenterostomy and subsequent cholangitis. Absence of the retrohepatic inferior vena cava shadow was noted on a chest x-ray film. At the time of transplantation, the absence of the retrohepatic inferior vena cava was confirmed. The portal vein was in a preduodenal location. Multiple splenic nodules were situated in the upper left quadrant (splenosis), and intestinal malrotation was present. This constellation of anomalies is not rare in biliary atresia.<sup>112</sup> Removal of the 405-gm liver was difficult because of multiple dense vascular adhesions and portal hypertension. The liver graft was taken from a large adult male donor whose exact weight is not known. A right tri-segmentectomy was done with an intact circulation, leaving the left lateral segment (weighing 700 gm) vascularized in the donor until the last possible moment. The graft was revascularized by connecting the donor left hepatic



**FIG 34.**

The first-known effort at use of a cut-down liver. The lateral segment of a large donor was transplanted to a 7½-year-old child who was dying of biliary atresia. The head of the child is to the left, and the legs are to the right. The lateral segment was too large, and the wound could not be closed. This operation was first used successfully by Bismuth of Paris and has been used extensively in France, Germany, Belgium, and the United States (see text).

vein end to end to the venous cloaca into which the diseased liver had drained. The donor left hepatic artery and left portal branch were anastomosed end to end to the recipient's common hepatic artery and portal vein, respectively. The donor left hepatic duct was anastomosed to a previously created Roux-en-Y jejunostomy.

After revascularization, the liver segment had immediate return of normal color and consistency. However, the fragment was too large to permit closure of the abdomen (Fig 34). Consequently, Dr. John Lilly covered the wound by suturing a sheet of Silastic-Marlex mesh to the peritoneum and fascia of the abdominal wound. A persistent bleeding diathesis occurred intraoperatively and subsequently. The child died 36 hours later, and at autopsy, the liver was relatively normal except for scattered focal infarcts. There was a 450-mL hemoperitoneum. Pulmonary *l*-isomerism and patent ductus arteriosus were also noted.

In this 1975 case, the fragment of liver that was retained still weighed almost twice as much as the excised native liver, dooming the effort to failure. In addition to the senior author of this monograph, members of the surgical team included many young surgeons whose continued academic activities are reflected in their current University appointments: John R. Lilly (Professor, University of Colo-



rado), C. W. Putnam, (Professor, University of Arizona), R. H. Bell (Associate Professor, University of Cincinnati), R. W. Beart (Professor, Mayo Clinic), M. Ishikawa (Professor, Tohoku University, Japan), and M. A. Haberal (Professor, Turkish Transplantation and Burn Foundation, Ankara).



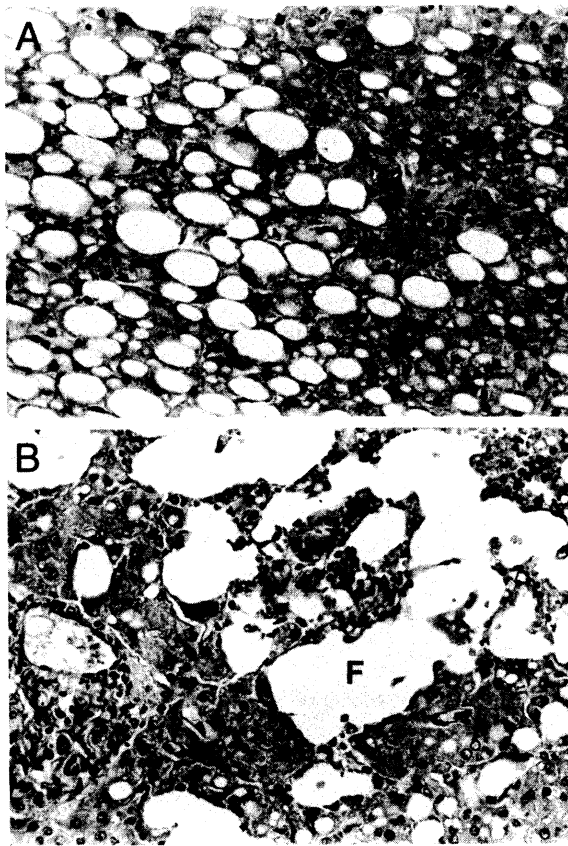
## EARLY GRAFT FUNCTION

The correction of preexisting liver function abnormalities begins intraoperatively if good graft function is obtained. When the graft fails completely to provide function, the only recourse is prompt retransplantation before cerebral edema and brain stem herniation occur.<sup>113</sup> Lesser degrees of graft injury can lead to renal failure, altered consciousness, a need for prolonged ventilatory support, ileus, and a host of other complications, which, even if they are not lethal, require protracted intensive care unit stays and generate astronomical hospital bills.<sup>114</sup> The penalties of primary dysfunction or nonfunction are so severe that much effort has been made to delineate the causes, to prevent these, to quickly quantitate the prospects of recovery, and to facilitate decisions about urgent retransplantation.

Since late 1987, the incidence of early graft failure necessitating retransplantation in the first 3 months or leading to death has been about 10%.<sup>52</sup> This incidence was down from 18% in the immediately preceding period.<sup>52, 113</sup> However, primary graft failure still occurs in 10% to 15% of cases.<sup>52, 115, 116</sup> There are four general reasons for graft failure, which are not necessarily mutually exclusive: (1) unrecognized liver disease in the donor, (2) a technically imperfect recipient operation, (3) ischemic injury of the graft, or (4) an immune event perioperatively. In Part II (*CPS*, March 1990) we will discuss the fourth factor, and will add a fifth factor, namely, endotoxemia, which is still speculative but too important to ignore as a possibility.

### *PREEXISTING DISEASE*

When a liver has primary nonfunction in spite of a seemingly perfect operation, it may have been diseased in the donor even though the tests used to screen donors were acceptable. Undetected chronic disease has been distinctly uncommon in livers that have passed through the donor screening process.<sup>117-119</sup> However, a few indisputable examples in which the donor livers had diffuse fatty infiltration (Fig 35) or other serious abnormalities have been reported.<sup>117, 120</sup> Rarely, an unrecognized malignancy can be transferred with the donor liver.<sup>121</sup>



**FIG 35.**

Transplantation of a liver with severe macrovesicular steatosis involving more than 80% of hepatocytes, as is shown in **(A)**, from a back-table biopsy predictably results in graft failure. After reperfusion **(B)** lysed hepatocytes release the fat (**F**, clear spaces), which contributes to microvasculature disruption with fibrin deposition and leukocyte sludging. (From Todo S, Demetris A, Makowka L, et al: *Transplantation* 1989; 47:903–905. Used by permission.)

The pathologist frequently is requested to evaluate a donor liver by frozen section before implantation because of gross physical alterations or suspicious agonal events in the donor. Gross inspection of the potential allograft by the pathologist is mandatory. Donor diseases recognized on frozen section in Pittsburgh have included metastatic carcinoma, diffuse regenerative hyperplasia, focal nodular hyperplasia, small noncaseating granulomas, severe steatosis, probable alcohol-induced injury, changes consistent with chronic active, persistent or nonspecific reactive hepatitis, and multiple small subcapsular infarcts. The livers with carcinomas, diffuse regeneration hyperplasia, and chronic active hepatitis have not been used. Those

with severe steatosis (see Fig 35) have also been routinely disregarded after several organs with similar changes were transplanted and failed.<sup>120</sup> Donor organs with nonspecific reactive hepatitis, small noncaseating granulomas, and other mild nonspecific changes are routinely used and have not caused problems. Usually, small focal nodular hyperplasia lesions are removed before implantation. In the absence of any of the obvious contraindications or severe ischemic injury, the pathologist is unable to predict the adequacy of organ function after transplantation based on frozen section light microscopy prior to the operation.

#### *TECHNICAL FAILURE*

Early retransplantation has been successful in less than one half of the cases when carried out in patients whose primary graft failure was caused by technical deficiencies.<sup>113</sup> This reflects in part the infections that quickly develop in or around a graft that is imperfectly transplanted as well as the rapidity of hepatic decompensation in many of the recipients.

Florid technical complications account for less than 10% of primary graft failures in adults compared with 30% in pediatric recipients.<sup>113</sup> With very small pediatric recipients, defined by a weight of less than 10 kg or by an age less than 1 year, technical complications have been a significant factor in a 35% 1-year mortality.<sup>122</sup> Vascular thrombosis has been a particularly troubling problem in these tiny recipients.<sup>122-124</sup>

Thrombosis of the hepatic artery or portal vein is usually classified as a technical error. Most technical errors are obvious, but subtle flaws in revascularization can be hard to diagnose. Suboptimal portal venous flow or reduced hepatic arterial flow has been found with electromagnetic flow meter studies.<sup>125-127</sup> In some of these cases, an unsatisfactory and ultimately correctable situation was not suspected before the flow determinations were obtained. A few patients have undergone emergency reconstruction of the thrombosed arteries.<sup>126, 128</sup>

When a graft fails because of arterial thrombosis, the pathologist may be able to find an underlying defect in the artery such as intraluminal mural flaps, devitalization of part of the wall, or intramural dissection. In a multivariate factor analysis in pediatric recipients,<sup>129</sup> the risk of arterial thrombosis was increased if the vessels were smaller than 3 mm, if the anastomoses had to be revised, or if aortic or iliac grafts were needed as "conduits" to the hepatic artery.

Portal vein thrombosis has been rare and usually occurs when the splanchnic venous bed of the recipient was altered by a previous operation, such as a portal-systemic shunt or splenectomy.<sup>130</sup> Unless

they are looked for, venous thrombi can be carried to the recipient in the portal vein of the liver graft, particularly if there has been a splenic injury in the donor.<sup>131</sup> Spontaneous resolution of a portal vein thrombosis has been reported.<sup>132</sup> However, early portal vein thrombosis usually requires retransplantation.<sup>130</sup> A few patients have been saved by immediate or delayed operation and secondary portal vein reconstruction.<sup>67, 133</sup> Two patients whose reconstructed portal vein thrombosed have had distal splenorenal shunts.<sup>134-136</sup> The first of these patients is still well 7 years after transplantation and 6 years after the shunt.<sup>134, 135</sup>

It is also true that hepatic artery thrombosis does not necessarily lead to graft loss. The event may be completely asymptomatic in 20% to 30% of cases.<sup>123, 137, 138</sup> Until Doppler ultrasound examinations were used routinely,<sup>139</sup> the diagnosis would not have been suspected in these recipients. In contrast, all of the syndromes that develop in symptomatic patients are serious and include primary nonfunction, regional septic hepatic infarction of a liver of which the viable portions may retain good function, bacteremia, abscess formation, rupture of the dearterialized ducts with bile peritonitis or with bile leakage, and biloma formation within the graft parenchyma (Fig 36).<sup>74, 123, 137, 138, 140-143</sup> Later, multiple intrahepatic biliary

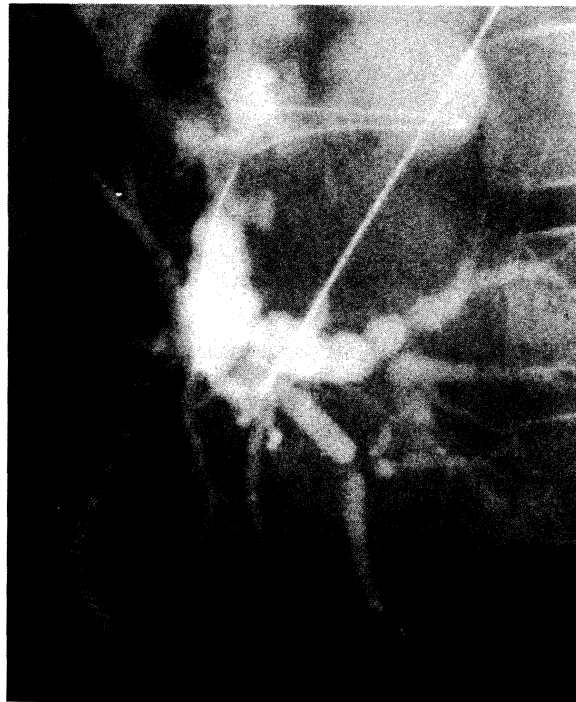


**FIG 36.**

Formation of a biloma within a dearterialized liver. Typically, patients with this complication have good liver function. It is possible to drain the biloma with a radiologically directed catheter, but retransplantation usually is necessary. (From Zajko AB, Campbell WL, Logsdon GA, et al: *Transplant Proc* 1988; 20[suppl 1]:607-609. Used by permission.)

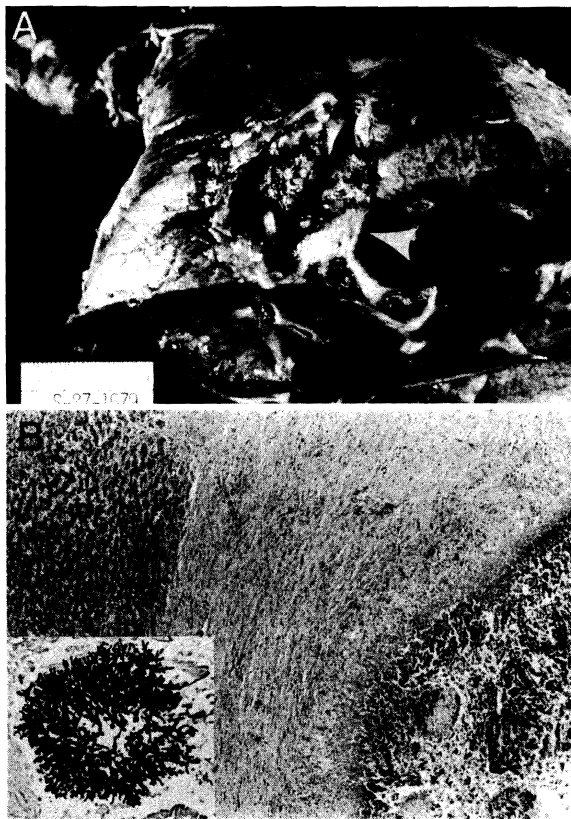
strictures resembling the lesions of sclerosing cholangitis may form (Fig 37).<sup>138, 141, 142</sup>

The diagnosis of hepatic artery thrombosis has been made much more frequently since the availability of Doppler ultrasound. Before then, arteriography was needed as a definitive step, but this was not commonly done. Needle biopsy is a rather insensitive method for establishing the diagnosis of hepatic artery thrombosis.<sup>144, 145</sup> The histologic changes can be quite variable and core needle biopsies are subject to more sampling error than usual. The findings may range from completely normal to frank coagulative necrosis. Marked perivenular hepatocellular swelling, cholangiolar proliferation, often with bile plugs, and acute cholangiolitis similar to that seen with "preservation injury" may also be observed. The pathologist should routinely search for microorganisms when necrotic tissue is encountered, since these foci frequently become seeded with bacteria and fungi (Fig 38).



**FIG 37.**

Multiple strictures in a patient whose hepatic artery clotted early. The recipient survived but ultimately developed cholangitis from multiple strictured and obstructive sites. The resulting appearance of the duct system has some resemblance to sclerosing cholangitis. (From Zajko AB, Campbell WL, Logsdon GA, et al: *Transplant Proc* 1988; 20[suppl 1]:607–609. Used by permission.)



**FIG 38.**

**A**, gross examination of a failed allograft with hepatic artery thrombosis often reveals necrosis of the hilar structures, including the connective tissue (*arrowhead*). **B**, microscopically, the large bile ducts are often necrotic, and the dead tissue becomes seeded with microorganisms, which was *Candida* in this case. (From Demetris AJ, Kakizoe S, Oguma S, Pathology of liver transplantation, in William JW [ed]: *Hepatic Transplantation*. Philadelphia, WB Saunders Co, 1990, pp 60–113. Used by permission.)

Since the hepatic artery is the sole direct supply of blood to the major bile ducts, intrahepatic ducts, hilar connective tissue, lymph nodes, and walls of the portal vein,<sup>146</sup> compromise to arterial flow frequently leads to selective necrosis of these structures (see Fig 38). In addition, an allograft may be more susceptible than non-grafted livers to this form of injury since it is devoid of the natural cascade type of arterial collaterals, at least in the early postoperative period. The areas prone to necrosis are not easily accessible to routine needle biopsy sampling. Therefore, biopsy monitoring of an allograft with a thrombosed artery may lead to a false sense of security.



### ***“Medical” Factors Contributing to Vascular Thrombosis***

Preoccupation about mechanical and technical causes of graft thrombosis is justified. However, so-called medical factors can contribute to or even make inevitable the thrombosis of a hepatic artery or portal vein. Overzealous correction of clotting defects during operation was shown long ago to predispose to vascular thrombosis in small children,<sup>147</sup> a lesson recently relearned with the use of fresh frozen plasma.<sup>129</sup> Polycythemia caused by transfusion is another iatrogenic risk factor.<sup>148</sup> The tendency of children to clot their vessels may be greater than in adults because of deficiencies in protein C and antithrombin and by defective fibrinolysis.<sup>149</sup>

An additional factor of unknown significance is the institution of cyclosporine therapy. This drug alters the prostanoid metabolism and other hemostatic processes of vascular endothelial cells.<sup>150–153</sup> Finally, a drastic reduction in hepatic blood flow is a well-known feature of rejection.<sup>154</sup> In a French clinical study, hepatic artery or portal vein thrombosis was associated with rejection more strongly than with any other definable factor.<sup>155</sup>

### ***Microvascular Injury***

Another factor making the new liver vulnerable to thrombosis during the perioperative and early postoperative periods is injury to the hepatic microvasculature from ischemia and cold preservation.<sup>156–159</sup> The denudation of the sinusoidal lining in preserved livers as assessed by light and electron microscopic studies is now known to be so extensive<sup>159</sup> that it is surprising that vascular thrombosis is not even more common than it is.

### ***ISCHEMIC INJURY***

It is not practical at present to measure in advance or even to estimate very accurately the ischemic injury during the events causing donor death, the procurement operation itself, and the period of formal cold preservation. The interval from cessation of donor circulation to cooling of the liver with preservation fluid is called *warm ischemia time*. The storage time after this plus the time to sew in the liver and restore its portal flow after removing it from an ice chest are termed *cold ischemia*. Under conditions of brain death pronouncement, and with modern techniques of multiple-organ procurement,<sup>23, 24</sup> there is virtually no warm ischemia.

Thus, almost all clinical reports equate cold ischemia with global ischemia. If this simplistic view were correct, the degree of organ damage would be a direct reflection of preservation time. The expected association can be demonstrated easily in controlled animal experiments<sup>36, 44, 56, 157–160</sup> but far less clearly in a clinical setting.

Part of the unpredictability could be caused by the variability of the time required to establish portal reperfusion and after this to restore the arterial supply. Rearterialization may be accomplished in some cases within 20 or 30 minutes after portal revascularization, but in others in which bleeding disrupts the desired routine, the interval can be many hours.

As described earlier, with the preservation techniques that were in clinical use through 1987, the safe preservation limits for human livers were set at 6 to 8 hours. These limits were conservative since dog livers could be stored for two or three times this interval after infusion with oncologically controlled electrolyte (Collins') solutions with a high potassium concentration<sup>36</sup> or with a plasma-like solution.<sup>44</sup> When the potassium-rich Euro-Collins solution was used to store human livers for 3 to 8 hours, there was no correlation at all between liver injury and preservation time as judged by a battery of liver function tests.<sup>52, 53</sup> Makowka and associates<sup>161</sup> and Miller and colleagues<sup>162</sup> made the additional perplexing observation that the condition of the donor was not important in influencing the outcome. Seemingly "unsatisfactory" cadaveric donors with poor blood gases, an unstable cardiodynamic state, or even moderately abnormal hepatic function tests provided livers that performed as well as organs removed from ideal donors. The same thing has been reported from the European liver registry.<sup>163</sup>

The fact that liver injury as judged by hepatic function tests, as well as graft and patient survival, has not had a significant association with preservation time does not mean that long storage times should be accepted lightly. Even with the UW solution,<sup>161</sup> very significant deterioration of graft quality has been demonstrated in controlled canine experiments between 1 and 24 hours of preservation.<sup>56</sup> Apparently, undefined factors in the heterogeneous human donor and recipient population are important enough to obscure the expected time/tissue damage relationship.

At present, the transplantation itself serves as the test by which the assessment of ischemic injury is made after the fact instead of prospectively. Intracellular pH, energy charge, mitochondrial function, and surrogate or direct measures of oxygen free-radical species in preserved liver tissue do not accurately predict graft quality in experimental animals.<sup>164, 165</sup> Instead, the ATP content of the preserved graft falls sharply even during the initial chilling infusion. Because it is the rapidity of ATP restoration after revascularization rather than its level before reperfusion that is discriminating as a prognostic sign, ATP measurements during preservation have not been thought to be helpful prospectively, with the exception of a single clinical report.<sup>166</sup>

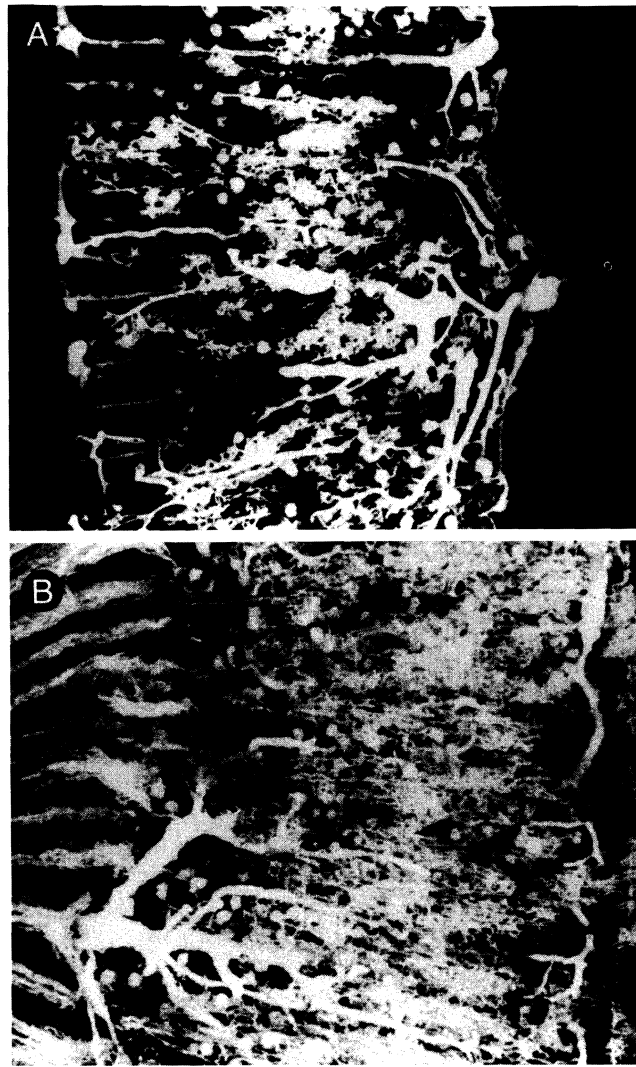
It may be that none of these metabolic tests are appropriate since they all reflect hepatocyte metabolism. This would seem logical

since in the past, it has been assumed that the parenchymal cells of whole organ grafts were the most vulnerable targets of ischemia. As was described in an earlier section, attention has shifted to the microvasculature, which not only may be the most exquisitely sensitive component of many whole organs but which also ensures (when injured) a perpetuation of parenchymal ischemic injury. For example, in studies of canine kidneys, Ueda and associates have demonstrated with a microphil technique the remarkable "pruning" of the terminal arteries and arterioles that can occur within 60 minutes after restoration of the renal arterial supply of inadequately preserved kidneys.<sup>167</sup> A devascularization is the consequence that is far less extreme in kidneys preserved with UW solution than in kidneys preserved with the Euro-Collins solution (Fig 39).

The sinusoidal endothelium of the liver is a unique microcirculatory bed. It lacks a well-defined basement membrane, is structurally specialized, forming large fenestrae to allow exchange of metabolites between the blood and hepatocytes, and is in close proximity to the Kupffer cells.<sup>168</sup> The cell swelling and subsequent damage that occurs during hypothermia are thought to be responsible for the focal areas of sinusoidal lining cell denudation observed ultrastructurally after cold preservation.

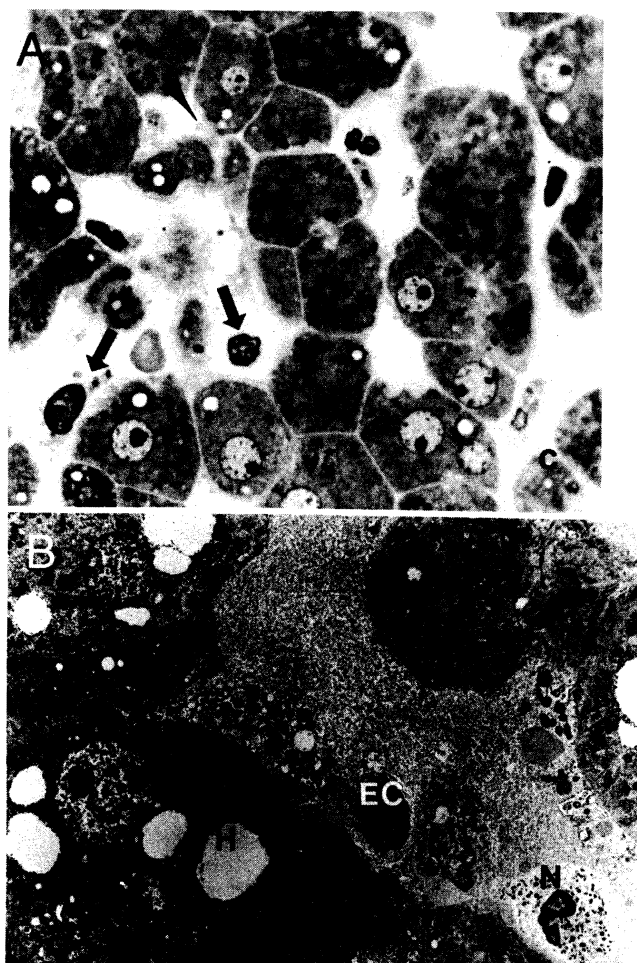
Destruction of the liver that occurs after reimplantation by the "reperfusion" mechanism is thought to be caused by two different but interrelated events.<sup>159, 169</sup> In the first, loss of the sinusoidal lining cells disrupts the architectural framework of the hepatic microvasculature, preventing adequate restitution of the blood flow. Instead of the antithrombogenic environment normally present in the sinusoids, exposure of the blood to coagulation stimulants results in fibrinogen activation and local clotting with trapping of red blood cells and leukocytes.<sup>159, 169</sup> This contributes to the circulatory blockade and fosters the accumulation of leukocytes. These cells likely serve as sources of tissue damaging oxidant (free radical) molecules, which is the second proposed pathway of destruction during reperfusion injury.

Protocol biopsies of human liver allografts obtained during back-table preparation and 1 to 2 hours after revascularization in the recipients have detailed the sequential histologic events that occur after reperfusion.<sup>169</sup> As would be expected, the vast majority of back-table biopsy specimens are essentially normal by light microscopic examination except for hydropic cell swelling. Sinusoidal lining cell integrity cannot be reliably evaluated on immersion-fixed, paraffin-embedded, and routinely stained sections. However, ultrastructural examination of the same biopsy specimens may show severe sinusoidal lining cell damage and denudation (Fig 40) like the changes observed in animals.<sup>156-159</sup> However, no specific histologic feature on the back-table biopsy specimen is able to predict postoperative or-



**FIG 39.**

Dissecting photomicrographs of renal vascular architectures filled with silicon rubber compound 1 hour after reperfusion of the grafts ( $\times 40$ ). **A**, 72-hour Euro-Collins group. Notice complete filling defect of subcapsular cortex and medulla. Patchy distribution of a vascular area, irregular and deformed pattern of interlobular artery and glomerulus can be seen. **B**, 72-hour UW group. The capillary networks of both cortex and medulla are fully filled with silicon rubber. (From Ueda Y, Todo S, Imventarza O, et al: *Transplantation* 1989; 48:913-918. Used by permission.)



**FIG 40.**

**A**, plastic-embedded sections of donor livers reveals that the sinusoidal lining cells bear the brunt of cold preservation injury. Note the endothelial cell denudation (*arrows*) with loss of the space of Disse. Hepatocytes usually show mild reversible changes such as a fatty vacuolization and bleb formation (*arrowhead*). **B**, ultrastructural analysis confirms the loss of sinusoidal endothelial cells, and leukocytes become directly adherent to hepatocytes (*EL* = endothelial cell; *L* = lymphocyte; *N* = neutrophil; *H* = hepatocyte) (From Kakizoe S, Yanaga K, Starzl TE, et al: *Hepatology* [in press]. Used by permission.)

gan function other than those that preclude organ use (see earlier discussion).

Within hours after reperfusion, livers that were minimally damaged during preservation show surprisingly few pathologic alterations. By contrast, zonal coagulative hepatocellular necrosis, either in the perivenular or periportal regions, accompanied by a brisk

neutrophilic exudate, and acidophilic bodies scattered throughout the lobule are signs of serious graft injury and harbingers of poor postoperative function in many instances (Fig 41). The evaluation of postperfusion injury can be influenced by the site of biopsy. It must be remembered that core needle biopsy specimens taken from the periphery of the organ may show more severe injury than the deeper parenchyma, and as always, the pathology findings should be interpreted in context with the complete clinical profile.

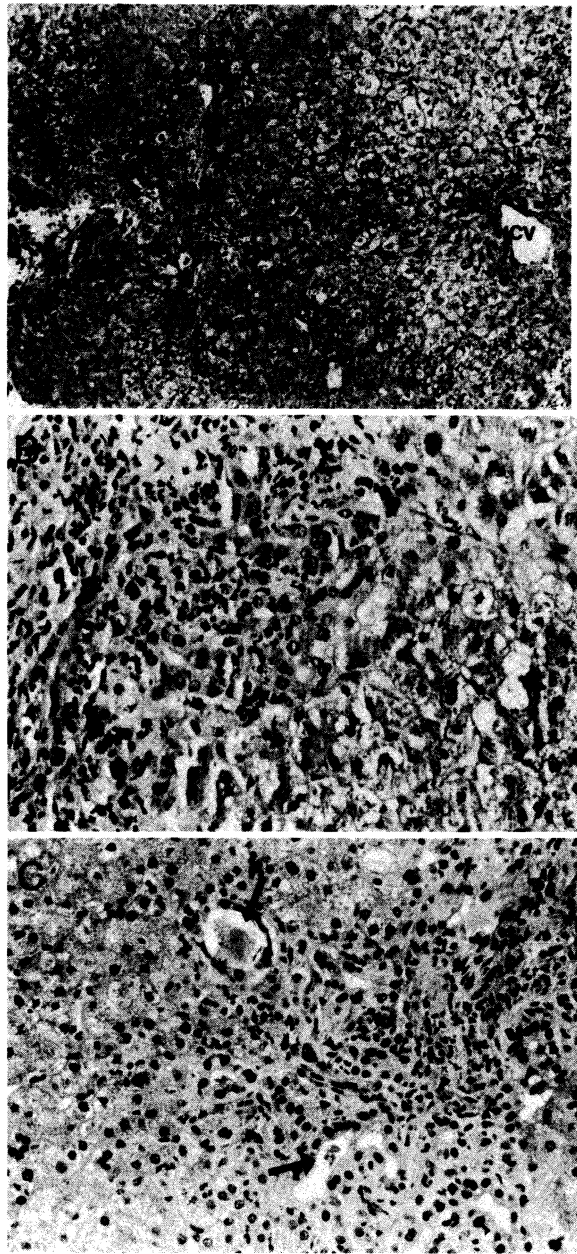
Once the liver is revascularized, quick assessment of its quality from metabolic studies is far more practical than a postperfusion biopsy. Measurements of blood amino acids clearance and study of other products of intermediary metabolism have been used to distinguish those patients whose new livers can and cannot be expected to recover.<sup>170-173</sup> However, one of the simplest of all signs, namely, bile production by the new liver, has long been recognized as the most important predictor of success after revascularization. Recent studies in animals<sup>174, 175</sup> and humans<sup>176</sup> have shown an almost perfect correlation between bile production, the rapidity of restoration of liver ATP levels after revascularization, and survival.

Next to bile production by the graft, restoration of good clotting in the recipient<sup>95-101</sup> and absence of lactic acidosis<sup>116, 170, 171</sup> are predictors of success. The coagulopathies that occur intraoperatively during liver transplantation are characterized by fibrinolysis, deficiencies of specific clotting factors and platelets, and consumption



**FIG 41.**

Zonal hepatocellular necrosis in a reperfusion biopsy, particularly when periportal in distribution (arrows), is a harbinger of poor postoperative function in many cases (PT = portal tract; CV = central vein). (From Kakizoe S, Yanaga K, Starzl TE, et al: *Hepatology* [in press]. Used by permission.)



**FIG 42.**

**A**, in the first few weeks after transplantation, grafts with mild ischemic injury show centrilobular hepatocyte swelling and hepatocanalicular cholestasis (*PT* = portal tract; *CV* = central vein). **B**, when the initial injury is more severe or periportal in distribution, cholangiolar proliferation and acute cholangiolitis are seen and represent attempts at repair, which, in most cases, is successful (see text). **C**, the structural changes and cholangiolar bile plugs (*arrows*) may persist for 1 to 2 months while the serum bilirubin level slowly declines.

of the clotting components.<sup>63, 95-101, 147, 177, 178</sup> Standard liver function tests during the following days almost always verify the accuracy of the simple intraoperative assessments of bile production and clotting.

Even organs severely damaged from preservation have the ability to completely recover after transplantation, both functionally and structurally. Biopsy specimens are often obtained at several-day intervals or weekly during the first 1 or 2 months in such patients, because clinically they can develop a prolonged cholestatic syndrome that does not resolve with increased immunosuppressive therapy.<sup>144, 179</sup> A fairly ordered sequence of events may be seen in such specimens.

The histologic evolution of repair depends on the degree of destruction.<sup>144, 145</sup> If the initial damage was relatively mild, lobular regeneration, as evidenced by hepatocellular mitoses and twinning of the plates, starts 2 to 3 days after transplant and is complete by 7 to 10 days. Mild perivenular hepatocanalicular cholestasis and cell swelling are also common features (Fig 42). If the damage is severe, and particularly if it is periportal in nature, florid cholangiolar proliferation ensues, which is invariably accompanied by neutrophils (i.e., cholangiolitis) and the hepatocellular regenerative changes mentioned earlier. These biopsy specimens are also marked by extensive cholestasis, both hepatocanalicular and cholangiolar, simulating large duct obstruction (see Fig 42). Total or near-total restitution of the liver is the usual outcome if the patient is well enough otherwise to permit the liver time enough to recover; this may take up to 2 months.<sup>144, 145</sup>

For the pathologist, the major differential diagnoses for the findings associated with preservation injury include large bile duct obstruction, sepsis, and hyperalimentation-induced injury. The histologic features used to rule out duct obstruction are reviewed in the section on biliary tract obstruction. Sepsis may be virtually impossible to separate with certainty. Finally, coexistent rejection is not uncommon in these patients and is recognized pathologically by the appearance of a predominantly mononuclear portal infiltrate with evidence of venous endothelial and bile duct damage (see the discussion of acute rejection pathology).



## PERIOPERATIVE IMMUNE EVENTS

If other explanations for primary nonfunction or dysfunction of the liver graft have been exhausted, host immune factors may be responsible. It is well known that human kidney<sup>180, 181</sup> and heart grafts<sup>182-184</sup> can be destroyed almost immediately by humoral antibodies in a process called *hyperacute rejection*. There have been no unequivocal examples of hyperacute rejection after clinical hepatic transplantation, supporting the widely held opinion that the liver is resistant to this kind of antibody mediated injury. Because of this resistance, liver transplantation has often been performed in spite of positive cytotoxic crossmatches against the donor<sup>185-189</sup> and in spite of ABO incompatibilities,<sup>190-193</sup> which because of the antigraft specificities of the ABO isoagglutinins would preclude renal or cardiac transplantation. Although the liver is resistant to humoral rejection, it is probable that humoral antibodies can cause severe graft damage in humans.

### WITH ABO-COMPATIBLE DONORS

The role and importance of cytotoxic antilymphocyte antibodies in causing nonfunction of liver grafts are not well delineated. These antibodies with antigraft specificity in kidney recipients are highly predictive of hyperacute rejection, particularly if the antibody is of the "warm" IgG variety.<sup>194</sup> The central event of hyperacute rejection of the kidney is occlusion of the graft microvasculature by rapidly sequestered formed blood elements and by clotting factors.<sup>195-198</sup> A striking feature of hyperacute renal rejection if this does not go promptly to completion can be the development of a consumption coagulopathy and, sometimes, fibrinolysis.<sup>196, 197, 199, 200</sup>

The association of hyperacute kidney rejection with cytotoxic antibodies directed against donor lymphocytes was first described by Terasaki and associates<sup>180</sup> and confirmed by Kissmeyer-Nielsen and co-workers.<sup>181</sup> At first, the simplistic view was that the cytotoxic antibodies themselves were directly responsible for injuring the endothelium of the microvasculature. However, it was soon realized that the process was far more complex, that the end result resembled the

Schwartzman reaction that can be produced in the kidneys of animals injected with endotoxin,<sup>195</sup> and that destruction of the organ probably took place through the action of mediators. At the time, little was known about soluble mediators of the inflammatory response, and most of these biologically potent substances had not yet been discovered. The possible role of these mediators in hyperacute humoral rejections has been summarized from a modern perspective by Makowka and colleagues,<sup>201</sup> and in a following section, a possible additional association of these mediators with recipient endotoxemia will be mentioned.

Hyperacute rejection of the liver was suspected after one of the first clinical attempts of orthotopic liver transplantation in a child whose graft developed hemorrhagic necrosis a few hours postoperatively.<sup>202</sup> The gross description of this liver was similar to the findings described many years later in rats<sup>203</sup> and in rhesus monkeys<sup>204</sup> sensitized with skin homografts and blood transfusions before orthotopic liver transplantation. However, experiments in rodents have also demonstrated the difficulty of inducing intense enough sensitization to reduce hepatic graft survival<sup>205, 206</sup> or else have shown that liver heterografts are rejected by heterospecific antibodies later and less violently than the heart and presumably other organs.<sup>206, 207</sup>

Such is the resistance of the liver to cytotoxic antibodies that a positive cytotoxic crossmatch should not preclude an effort at liver transplantation. It also is becoming evident that accelerated (possibly humoral) rejection of liver grafts can occur.<sup>208-210</sup> However, the process develops more slowly than with the kidney and presumably other organs, it may be reversible, and it is not strongly associated with the antigraft antibodies that are being measured in routine typing laboratories.<sup>208</sup> A progressive and severe coagulopathy developing shortly after hepatic revascularization should arouse suspicion of an accelerated rejection, even if there has not been a positive cytotoxic antibody crossmatch.<sup>208</sup>

The resistance of the liver to hyperacute rejection from lymphocytotoxic antibodies is thought to be the result of several factors. The most important of these may be the dual afferent blood supply, a sinusoidal network coated with Kupffer's cells rather than a capillary microvasculature,<sup>168</sup> secretion of soluble major histocompatibility complex (MHC) antigens into the circulation,<sup>211-213</sup> and nontoxic absorption of alloantibodies or immune complexes by the Kupffer cells.<sup>169, 214-219</sup> The liver receives an afferent blood supply from both the hepatic artery and portal vein, and compromise to either results in compensatory flow in the other, presumably protecting the liver from ischemic injury.<sup>168</sup> Most of the microvasculature network of the liver is sinusoidal, which is lined by widely spaced (fenestrated) endothelium with no underlying basement membrane.<sup>168</sup> In contrast, both the heart and kidney have an arterial end organ blood supply

with only a capillary microvasculature, which, when occluded, results in ischemic necrosis. The only capillary microvasculature of the liver is that which derives from the hepatic artery and exclusively supplies the hilar structures and biliary tree. Occlusion of this system may result in a more limited form of graft injury (biliary) rather than total organ failure.

The lymphocytotoxic antibodies present in human or animal recipients of liver grafts disappear from the serum shortly after liver grafting.<sup>214, 215, 217, 219</sup> In fact, Houssin and associates<sup>214</sup> quite elegantly demonstrated in rats that prior liver allografting is able to protect extrahepatic (heart) grafts from undergoing hyperacute rejection.<sup>214-216</sup> Both a strong donor-specific and weaker nonspecific third party protective effect is seen.<sup>214-216</sup> Fung and colleagues have shown that liver allografts can protect kidney allografts from the same donors in presensitized humans and prevent hyperacute rejection.<sup>217</sup> They documented the disappearance of donor-specific anti-class I lymphocytotoxic antibodies from the recipient circulation shortly after transplantation.<sup>217, 219</sup> However, this protective effect is not always seen and can be overridden in animals<sup>203, 204</sup> and possibly humans.<sup>184, 208</sup> In animals, it was noted that intense sensitization protocols are required to overcome this effect.<sup>203, 204</sup> In humans, at least two recipients have hyperacutely rejected kidney grafts after they had received liver allografts from the same donor less than 1 day prior.<sup>208</sup> These cases have served as prototypes for the recognition of antibody-mediated rejection in the liver.

It is known that human and rat livers secrete soluble (class I MHC) antigens that presumably bind to and neutralize the circulating antibodies.<sup>211-213</sup> Gugenheim and co-workers have also shown in rats donor specific absorption or binding of the lymphocytotoxic antibodies and donor-specific cytotoxic T lymphocyte (CTL) by non-parenchymal cells of the liver.<sup>215, 216</sup> Kupffer cell blockade suppresses this protective effect. It also appears that Kupffer's cells may be involved in the neutralization of lymphocytotoxic antibodies in humans,<sup>169, 218</sup> either directly or indirectly, by binding immune complexes. Therefore, the liver probably acts as a "sink" for the deposition of the lymphocytotoxic antibodies, immune complexes, and perhaps CTLs. Whether this deposition is toxic or not may depend on the antibody class and titer and on the activity of the Kupffer cells at the time of challenge.

Knechtle and associates have recently shown that hyperimmunized rats hyperacutely reject livers within hours after transplantation.<sup>203</sup> Rat transplantation may not be the ideal model to study this phenomenon since, in most instances, no attempt is made to reconstruct the arterial supply. Gubernatis and colleagues were able to demonstrate early antibody-mediated rejection in presensitized rhesus monkeys.<sup>204</sup> The sensitized animals rejected the livers at an

average of 2.5 days compared with the mean graft survival of 26 days in unsensitized controls. Routine and immunopathologic studies of these grafts that had been rejected in an accelerated fashion demonstrated immunoglobulin deposits, arteritis, and ischemic necrosis, typical of that seen with hyperacute rejection of other organs. However, an extreme level of presensitization was required (multiple skin grafts and donor blood transfusions), which may not reflect most clinical situations where a positive lymphocytotoxic crossmatch is encountered. Furthermore, the antibodies apparently causing the damage in the animal experiments mentioned earlier<sup>203, 204</sup> were not well characterized. Whether this protective effect can be overridden by high-titer lymphocytotoxic antibodies in humans is not clear at present. If it does occur, routine lymphocytotoxic crossmatch results are unable to predict the phenomenon beforehand. The only apparent correlation between the pretransplant crossmatch and early postoperative events is a requirement for an increased number of platelet and blood transfusions.<sup>220</sup>

#### *WITH ABO-INCOMPATIBLE DONORS*

Although ABO-incompatible liver transplantation can be done in the event of extreme need,<sup>190-193</sup> the risk is increased.<sup>190-193, 221, 222</sup> Isoagglutinin fixation has been demonstrated in the microvasculature of ABO-incompatible liver grafts in a collection of cases in which hemorrhagic infarction occurred five times more frequently than with ABO-compatible grafts.<sup>221</sup> There have been several similar case reports of hemorrhagic infarction.<sup>193, 222</sup> Minor blood group antibody systems (Lewis) do not appear to influence graft survival.<sup>223</sup> Unexpectedly, ABO-identical grafts have done better than ABO-compatible but nonidentical organs, and O recipients did better in both the incompatible and nonidentical situations.<sup>190, 224</sup>

The prototype of antibody-mediated rejection of the liver is often, but not invariably, encountered when the major ABO blood group barriers are breached.<sup>221</sup> The syndrome that occurs is the liver equivalent of "hyperacute rejection," but in most instances it develops more slowly than is seen in heart or kidney grafts. The organs initially reperfuse well and produce bile. A change in the color or consistency may or may not be noted by the operative surgeon before abdominal closure, and difficulty in achieving hemostasis is not uncommon. During the first several posttransplant days,<sup>221</sup> the patients experience a relentless rise in liver injury test results. Angiograms performed to rule out arterial thrombosis may reveal diffuse luminal narrowing, consistent with vascular spasm. Eventually, hepatic failure ensues, which is manifest by wound site bleeding and encephalopathy, and retransplantation becomes necessary. Appearance of the organ at the time of reoperation is similar to that of other

organs undergoing hyperacute rejection. They are often enlarged, cyanotic, and mottled with areas of necrosis. The capsule may be ruptured and bleeding from the liver surface can be observed.

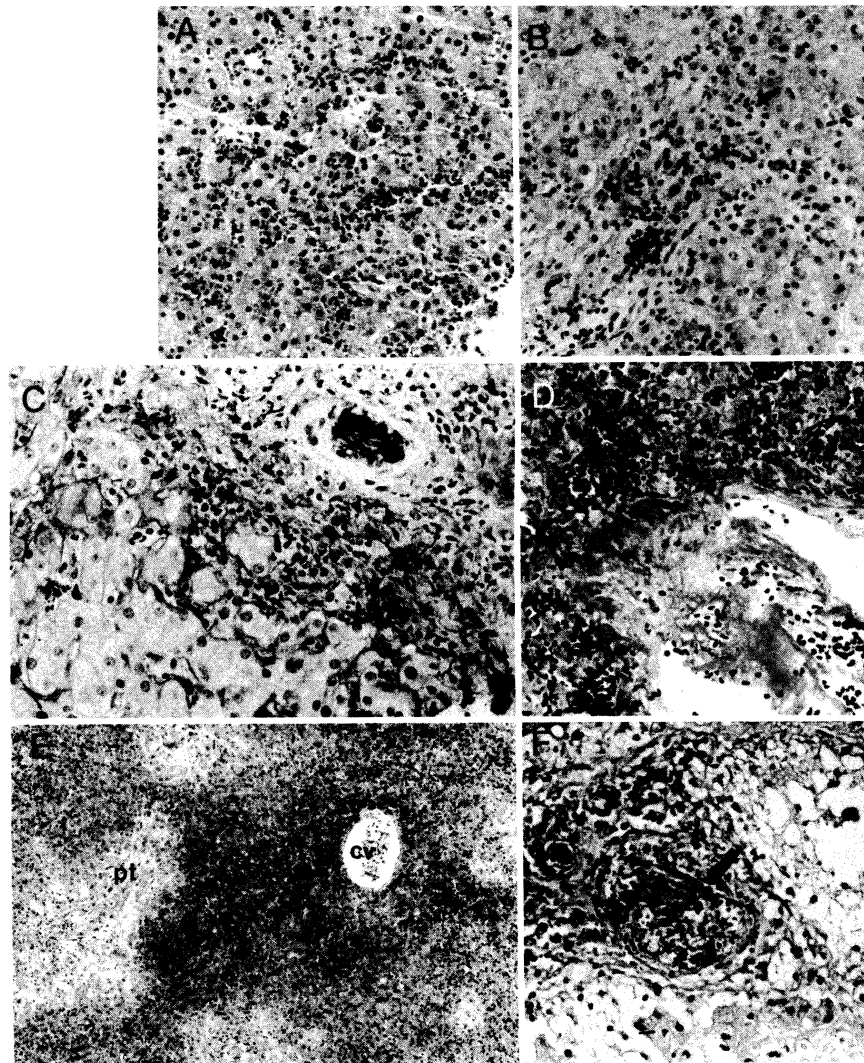
Needle biopsy evaluation during the development of antibody-mediated rejection demonstrates a progression of findings (Fig 43\*).<sup>221</sup> Samples taken within hours after reperfusion show prominent red blood cells sludging, clustering of neutrophils, and fibrin deposition in the sinusoids. Focal hemorrhage into the space of Disse, hepatocellular cytoaggregation, and single-cell acidophilic necrosis then follow. Small clusters of hepatocytes undergoing coagulative necrosis, red cell congestion, and hemorrhage appear in samples taken 1 to 2 days later. The areas of necrosis may not demonstrate any particular zonal distribution. Portal and central veins often shown partial fibrinoid degeneration of the wall, with the attachment of a fibrin aggregate, which extends in a flamelike fashion into the lumen. Arteries are usually less severely affected than the veins; endothelial cell hypertrophy, endothelial denudation, and focal fibrin thrombi are common findings. Intimal neutrophilic or necrotizing arteritis (or both) with medial inflammation can be seen on occasion (see Fig 43). Cholangiolar proliferation as a sign of regeneration is recognizable by 2 to 3 days, and the histologic features at this point may be quite difficult, if not impossible, to separate from preservation injury. Thereafter, progressive patchy hemorrhagic infarction of the organ occurs.

Immunofluorescence and immunoperoxidase staining done during the development of the syndrome will often reveal diffuse sinusoidal, venous, and arterial deposition of IgG and IgM, C1q, C3, and occasionally C4 (see Fig 43). However, only focal patchy deposition of IgM and C1Q will be detected in the failed organs. This change in the distribution of deposition is presumably because of rapid catabolization of the immune deposits.

A similar clinicopathologic syndrome may occur in ABO-compatible situations when no preformed lymphocytotoxic antibodies are present.<sup>208, 221, 225</sup> It is likely that other immunologic and nonimmunologic insults are capable of triggering intravascular coagulation and the cascade of events that occur within the liver, which result in hemorrhagic necrosis.<sup>184</sup> Therefore, a diagnosis of hyperacute or humoral rejection in the liver should be based on a complete clinicopathologic evaluation of a suspicious case, during which other nonimmunologic causes of graft failure are reasonably excluded.<sup>221</sup> In addition, several other criteria should be fulfilled (Table 2),† including demonstration of a presensitized state in the recipient, consistent light and immunofluorescent microscopic findings, and the

\*Figures 1–42 appear in Part I.

†Table 1 appears in Part I.



**FIG 43.**

Sequential histopathologic events during antibody-mediated liver allograft rejection. **A**, immediately after reperfusion, RBCs and neutrophils stuff the sinusoids. **B**, 1 to 2 days later, small clusters of hepatocytes undergo coagulative necrosis, and portal neutrophilia may be seen (*arrow*). **C**, immunoglobulin and complement components are usually detected diffusely throughout the hepatic vasculature early in the course of events, as shown here (immunoperoxidase for IgM), but may be harder to find later on. **D**, partial fibrinoid degeneration of the veins and arteries with intraluminal thrombi are the most characteristic vascular findings. **E**, eventual graft failure is due to widespread hemorrhagic necrosis without much of an inflammatory infiltrate (*pt* = portal tract; *cv* = central vein). **F**, necrotizing and/or neutrophilic arteritis (*arrow*) can be seen, as illustrated here but is found in a minority of cases. (From Demetris AJ, Jaffe R, Tzakis A, et al: *Am J Pathol* 1988; 132:489–502. Used by permission.)

**TABLE 2.**

Criteria for the Diagnosis of Hyperacute  
(Humoral) Rejection of Human Liver  
Allografts

- 
1. Early graft failure (usually 1–2 weeks after transplant) with no alternative clinical or pathologic explanation
  2. Consistent routine light and immunofluorescence microscopic findings
  3. Demonstration of a presensitized state in the recipient\*
  4. Presence of donor-specific antibodies in an eluate from the failed graft

\*Not necessarily lymphocytotoxic antibodies detected in conventional assays.

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presence of donor-specific antibodies in an eluate from the failed graft. Fulfillment of such a “Koch’s postulate” for hyperacute rejection may be overly restrictive, since there are antibody systems outside the ABO and lymphocytotoxins that have been associated with hyperacute rejection.<sup>183</sup> However, adherence to these criteria will add to the predictive value of screening for antibody systems in the future.

Primary nonfunction of a liver homograft without an obvious explanation should suggest that the new organ may have placed into an environment that is hostile because of immunologic or perhaps nonimmunologic factors. The prompt destruction of hepatic retransplants in patients whose first liver grafts have been lost for inadequately explained reasons has been seen in several centers with large experience, causing the word of mouth descriptive term “liver eaters” to be applied to such recipients<sup>208</sup> in the absence of an explanation for their behavior.

#### *THE QUESTION OF ENDOTOXEMIA*

The inability to predict the perioperative outcome after liver transplantation with prognostic premonitors such as quality of donor, time of ischemia, and even the presence of antidonor cytotoxic antibodies has led to a search for other factors. Endotoxemia is one of the most interesting of these possible factors.

Endotoxin is a macromolecular component of the cell wall of gram-negative bacteria. Its most specific and active component is lipid A.<sup>226</sup> However, it has been increasingly recognized that protein and polysaccharide components of the molecule can influence its potency and specificity.<sup>227, 228</sup> Because gram-negative bacteria are in-

digenous to the gastrointestinal (GI) tract, an enteric source must be suspected when symptomatic endotoxemia is diagnosed.<sup>228</sup>

There is evidence that small quantities of endotoxin can cause serious or lethal syndromes in animals and humans.<sup>229, 230</sup> However, a cause and effect relationship may be difficult to establish in specific situations.<sup>231</sup> One reason is that the presence of endotoxin, even in large amounts, may not necessarily be associated with symptoms.<sup>232</sup> Another reason is that the responses elicited by endotoxin are not specific or unique.<sup>227, 233</sup> Endotoxin can induce the release of a complete spectrum of biologically active substances, including soluble mediators of the inflammatory response and cytokines (Table 3). Activation of the individual mediators, including the cytokines, is induced by a direct effect of the endotoxin on complement, macrophages, monocytes, and other formed blood elements, including lymphocytes and endothelial cells (see Table 3).

The soluble mediators that can be released into the circulation or locally theoretically could have devastating physiologic effects (see Table 3), including fever, shock, vasodilatation, vasoconstriction, coagulation disorders, smooth muscle contraction, endothelial injury, chemotaxis, tissue necrosis, and even neuropsychiatric changes. In addition, the majority of the mediators have immunoregulatory functions, predominantly augmenting either cellular or humoral immunoreactivity, or both (see Table 3). This latter feature of the soluble mediators may be particularly important in the context of transplantation. What results from exposure to endotoxin could be a combination of the effects of many or even all of the mediators. The difficulty of interpretation is compounded by the fact that many factors other than endotoxin can activate the mediators and by the variable functional interactions between the mediators themselves.<sup>227, 234</sup> Immune responses could be interlocking with or simulate endotoxin, as was speculated nearly 20 years ago in a report on hyperacute rejection of the kidney.<sup>195</sup> In that article, the possibility was discussed that endotoxin might be able to destroy kidney grafts in a way analogous to the hyperacute rejection caused by cytotoxic antigraft antibodies. At that time, little was known about soluble mediators and cytokines. Now, it is easy to conceive that these substances, including those that are immunoregulatory (see Table 3), could participate in an endotoxin-initiated injury, a humoral immune reaction, or a combination of these.

The liver plays a control role in the modulation of endotoxin. Intravenous (IV) endotoxin is removed mainly by the Kupffer cells of the liver.<sup>218, 235, 236</sup> Not only is this detoxification system absent during the anhepatic phase of transplantation, but there is a subsequent transformation in the graft whereby donor Kupffer's cells are replaced with macrophages of recipient origin<sup>237, 238</sup> that may be accelerated in pathologic states.<sup>239</sup> In addition, the transplanted liver is exposed to intestinal bacteria that reach the liver in splan-



**TABLE 3.**

Soluble Mediators (Including Cytokines) That are Activated by Endotoxin\*

|                                  | Description of Mediator   | How Endotoxin Initiates Mediator Production   | Physiologic Consequences   |
|----------------------------------|---|---|--|
| Anaphylatoxins<br>C3a and C5a    | Cleavage products of C3 and C5 complement   | Activates serum complement (classical and alternative pathways)   | Vasodilatation, smooth muscle contraction, mononuclear cell and neutrophil chemotaxis, immunomodulation of humoral response  |
| Prostaglandins                   | Cyclo-oxygenase pathway from arachidonic acid   | Activates macrophages and monocytes   | Vasodilatation, activates or collaborates with other mediators, modulates macrophage effect or function  |
| Leukotrienes                     | Lipoxygenase pathway from arachidonic acid  | Activates macrophages and monocytes   | Vasoconstriction, activates or collaborates with other mediators, modulates macrophage effect or function  |
| Platelet-activating factor (PAF) | Cell derived (from platelets, neutrophils, basophils, mononuclear phagocytes, endothelial cells), lipid mediators | Binds to platelets, neutrophils, etc., with mediator release  | Platelet aggregation, neutrophil degranulation, smooth muscle contraction, increased vascular permeability, hypertension, tissue necrosis, modulates endothelial cell function |
| Tissue factor                    | Glycoprotein from monocyte or macrophage cell surfaces  | Activates factor XII (intrinsic coagulation pathway) stimulates mononuclear cells (extrinsic coagulation pathway) | Microvascular thrombosis   |

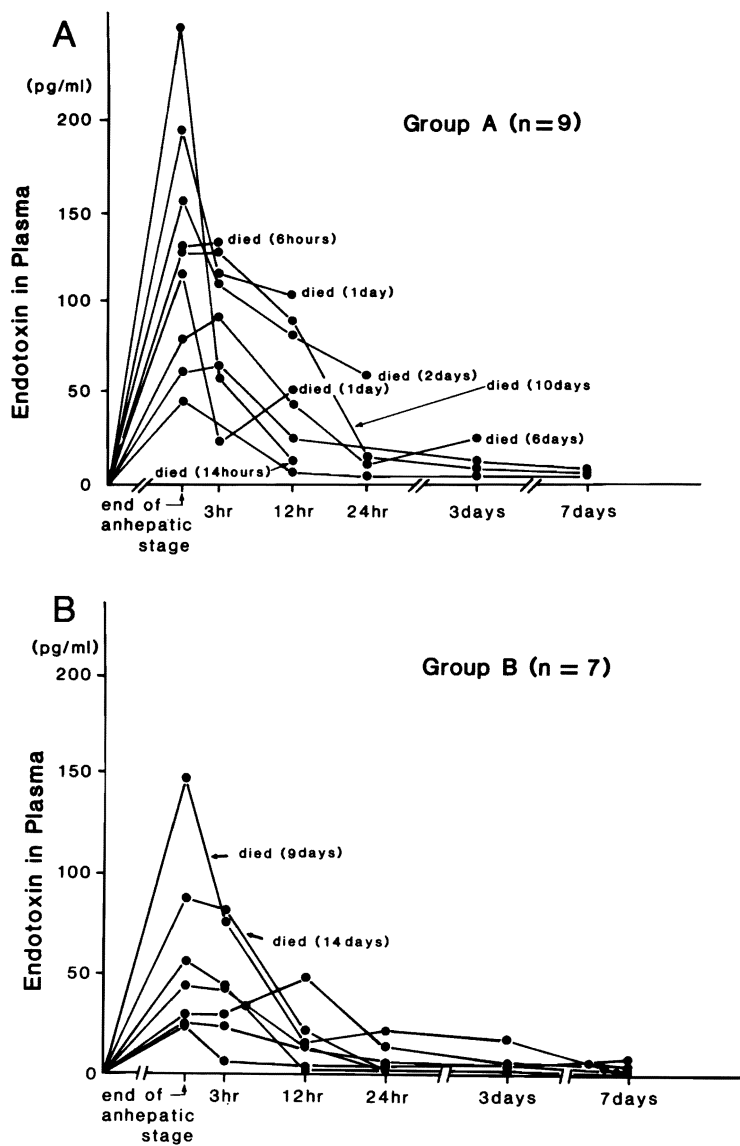
(Continued.)

TABLE 3 (cont.).

|                                   | Description of Mediator                                       | How Endotoxin Initiates Mediator Production  | Physiologic Consequences  |
|-----------------------------------|---|--|---|
| Interleukin 1 (IL-1)              | Family of immunoregulator cytokines produced by monocytes     | Stimulates mononuclear phagocytes and other cells  | Fever, lymphocyte activation, coagulation, endothelial cell adhesiveness, enhancement of T- and B-cell immunity secondarily activates PAF, arachidonic acid products, etc.          |
| Tumor necrosis factor (cachectin) | Product of activated macrophages                              | Activates macrophages production   | Fever, induces IL-1 from mononuclear and endothelial cells, cytotoxic to tumor cells, amplifies microvascular coagulation   |
| Colony-stimulating factor         | Heterogenous glycoproteins from macrophages and B lymphocytes | Induces production by macrophages and B lymphocytes  | Stimulates proliferation and differentiation from marrow-derived precursor cells, activates mature macrophages to produce other mediators   |
| Interleukin-2                     | Lymphokine from activated T lymphocytes                       | Complex pathway by stimulation of IL-1 and IL-2 production from lymphocytes and IL activation of interferon alpha production <sup>†, 22a</sup> | Increases antibacteria-1 and antitumor activity of macrophages, increases expression of Fc receptors, augments other immune responses, amplifies endotoxin effects (?viscous cycle) |
| Endorphins                        | Endogenous opioids  | Unknown, could stimulate mononuclear cells   | Hypotension, analgesia, behavior changes, immunoregulation (enhancing and suppressing)  |

\*Modified from Morrison DC, Ryan JL: Endotoxin and disease mechanisms. *Annu Rev Med* 1987; 38:417-432. Used by permission.

†Interferon alpha and beta are induced by endotoxin directly from B lymphocytes and macrophages.



**FIG 44.**

Endotoxin levels in picograms per milliliter in 16 consecutive dogs submitted to orthotopic liver transplantation. Note that every animal had an endotoxin level increase by the end of the anhepatic phase (groups A and B) and that this was less in animals who had an antibiotic bowel prep in advance (group B) compared with those who did not (group A). The endotoxemia lasted for many hours or even days afterward. (From Miyata T, Todo S, Imventarza O, et al: *Transplant Proc* 1989; 21:3861–3862. Used by permission.)

nic blood and through the biliary tract and then "leak" through to the systemic circulation.<sup>240, 241</sup> For all of these reasons, endotoxin would be a prime suspect in looking for causes of perioperative morbidity.

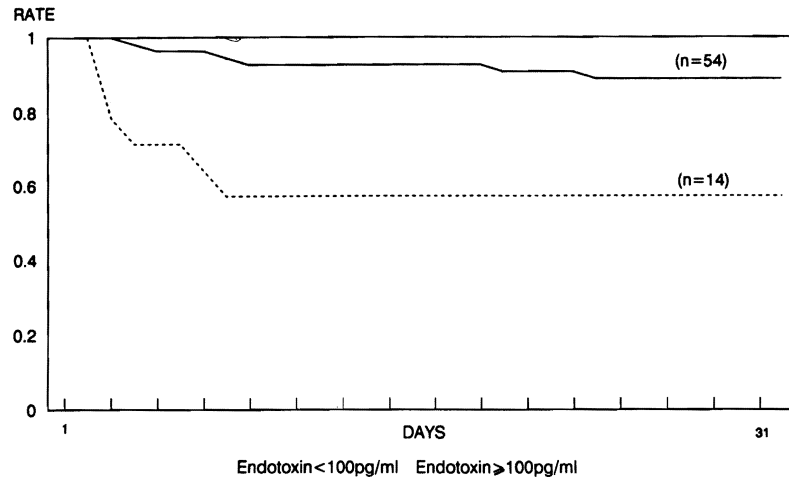
However, the obvious possibility that endotoxin was responsible for perioperative problems after liver transplantation was not investigated until recently. The detection of endotoxin in plasma was unreliable,<sup>242, 243</sup> and only a qualitative assay was available.<sup>244</sup> The chromogenic substrate method developed by Iwanaga and colleagues<sup>245</sup> in 1978 paved the way to a sensitive quantitative assay of endotoxin. Using this principle, Obayashi and associates introduced a novel method based on the combination of plasma treatment with perchloric acid and the chromogenic substrate method,<sup>246, 247</sup> making possible meaningful correlations between endotoxemia and clinical syndromes such as coagulopathy with hemorrhage, cardiovascular collapse, primary nonfunction of hepatic grafts, acute renal failure, respiratory insufficiency, and multiple-organ failure.

The first studies of endotoxemia in liver transplantation were reported by Miyata and co-workers in 1989, using the new analytic techniques to study 16 normal healthy dogs before and after liver replacement.<sup>248</sup> Nine of the animals had a preoperative bowel prep with oral neomycin. After operation, all of the dogs were treated with cyclosporine. All 16 of the animals had a significant increase in plasma endotoxin levels, which peaked at the end of the anhepatic period and remained elevated for several days. The magnitude of the rise was significantly lower in dogs with an antibiotic bowel prep (Fig 44), and these dogs had better survival.

In addition, plasma endotoxin levels in nearly 100 liver transplant patients were measured before transplant, at the end of the anhepatic phase, and on postoperative days 1, 3, and 7. In this study by Yokoyama and colleagues, the presence of high endotoxin levels preoperatively and at the end of the anhepatic period was associated with graft failure and a high mortality (Fig 45).<sup>249</sup>

Patients with primary nonfunction of their transplants typically had severe endotoxemia. In nine patients with primary nonfunction, most of the endotoxin levels were only moderately elevated preoperatively. However, large further increases occurred in the plasma in seven of the nine patients by the time the new livers were revascularized. The livers acted as if they had been revascularized in a hostile environment. Only two of the nine patients had positive cytotoxic crossmatches with their donors, but all nine of the livers behaved as if hyperacute rejection had occurred.

Thus, endotoxemia could be a cause rather than an effect of perioperative graft loss, serious morbidity, and increased mortality. With the Cox proportional hazards model, the most powerful indepen-



**FIG 45.**

Correlation of endotoxin level at the end of the anhepatic phase with graft survival for 68 primary transplantations. (From Yokoyama I, Todo S, Miyata T, et al: *Transplant Proc* 1989; 21:3833–3841. Used by permission.)

dent factors associated with graft death in the study by Yokoyama and colleagues were endotoxemia greater than 100 pg/mL at the end of the anhepatic period, lactate level greater than 10mM/L at the same time, and serum glutamic pyruvic transaminase (SGPT) level greater than 200 IU/L preoperatively.<sup>249</sup> These exceeded in importance the degree of recipient illness, graft ischemia time, duration of anhepatic phase, cytotoxic crossmatch, and amount of blood transfusion.

In a further study of the patients who underwent primary transplantation, Miyata and associates showed that there was a strong correlation between the endotoxemia at the end of the anhepatic phase and the need for perioperative platelet transfusions, ventilator dependency postoperatively, and 1-month mortality.<sup>250</sup>

If endotoxemia can be shown to be a negative factor in the transplantation of the liver or other organs, therapeutic strategies might be devised to prevent this complication. Possibilities could include the use of antiendotoxin monoclonal antibodies<sup>251</sup> or, less specifically, the control of the gram-negative intestinal flora with antibiotics as described by Weisner and co-workers.<sup>252</sup> Polymyxin B is an antibiotic with a strong antiendotoxin activity.<sup>253</sup> An alterna-

tive way to use polymyxin B would be as part of an impregnated matrix<sup>254</sup> to which blood from the extracorporeal bypass could be exposed.

The studies done so far have been concerned primarily with recipient endotoxin. However, endotoxin also could adversely affect the liver and other organs of brain-dead donors, particularly if these are victims of severe trauma.<sup>229</sup> In a small group of six cadaveric donors, plasma endotoxin levels in two of the six were abnormally elevated, in the 10 to 20 pg/mL range.<sup>249</sup> More investigations on the matter of donor endotoxin are planned.

## PREVENTION OF REJECTION

At the time orthotopic liver transplantation was first studied in Boston<sup>6</sup> and Chicago<sup>8</sup> beginning in the summer of 1958, the only known technique for immunosuppression was with total body irradiation. Attempts were made in 1959 to influence rejection by irradiating either the canine liver donors or their recipients with 1,400 rad. Neither approach was helpful, and in fact, recipient irradiation led to 100% mortality. The results were so poor that they were not published until 1962.<sup>255</sup>

The possibility that there was an immune barrier to successful transplantation of tissues and organs apparently was not part of the consciousness of early clinicians or, for that matter, of most basic scientists. This realization awaited the classical studies of Medawar with rabbit skin grafts.<sup>256</sup> Appreciation by Medawar that rejection was an immunologic phenomenon made inevitable almost everything that followed. The deliberate depression of immunologic reactivity became feasible theoretically when total body irradiation<sup>257, 258</sup> and adrenal cortical steroids<sup>259</sup> were shown to be immunosuppressive. The next great step was the introduction of thiopurine compounds, 6-mercaptopurine and its imidazole derivative azathioprine, which inhibited heterohemagglutinin formation in mice,<sup>260</sup> responsiveness to foreign proteins in rats,<sup>261</sup> and rejection of skin and renal grafts in rabbits, rats,<sup>262, 263</sup> and dogs,<sup>264, 265</sup> respectively.

The foregoing laboratory research proved inapplicable to organ replacement in humans. Complete control of rejection with a single agent rarely was achieved without lethal side effects in either animals or humans, as exemplified by the historically important trials with total body irradiation<sup>266</sup> as well as by early trials with 6-mercaptopurine and azathioprine.<sup>267-271</sup> Hopeful signs from the clinical experience through 1962 were footnotes to an otherwise dreary catalogue of failures. In 1961, Burnet, a Nobel laureate with Medawar the preceding year, wrote in the *New England Journal of Medicine*<sup>272</sup>:

Much thought has been given to ways by which tissues or organs not genetically and antigenetically identical with the patient might be made to survive and function in the alien environment. On the whole, the present outlook is highly unfavorable to success. . . .

## THE HUMAN KIDNEY TRANSPLANT PROTOTYPE

Liver transplantation at first was a passive partner in the development of immunosuppressive techniques. Whatever the current practice was in clinical renal transplantation was passed on for secondary application to the extrarenal organs. The modern era of transplantation was entered when it was realized that azathioprine and prednisone had at least additive, and possible synergistic, effects.<sup>273</sup> With the use of living-related donors, renal transplantation became overnight a practical means of treating renal failure.<sup>273, 274</sup> There are only 23 patients left in the world from this early era (Table 4), all having been given kidneys from blood relatives.<sup>275</sup> Other multimodality techniques followed.

The most important new variable between 1962 and 1978 was the adjuvant use of antilymphocyte globulin (ALG) added to azathioprine (or to cyclophosphamide) and steroids.<sup>276</sup> Ultimately, it became possible to produce more potent and specific ALGs<sup>277</sup> with the hybridoma techniques discovered by Kohler and Milstein.<sup>278</sup> However, from 1963 to 1979 with any of the methods available, truly acceptable results were obtained only with renal transplantation from consanguineous donors. Candidates for liver transplantation were faced with the bleak prospect of receiving a nonrelated (cadaveric) graft.

The situation changed drastically for recipients of all kinds of cadaveric organs, including the liver (Fig 46), with the disclosure by

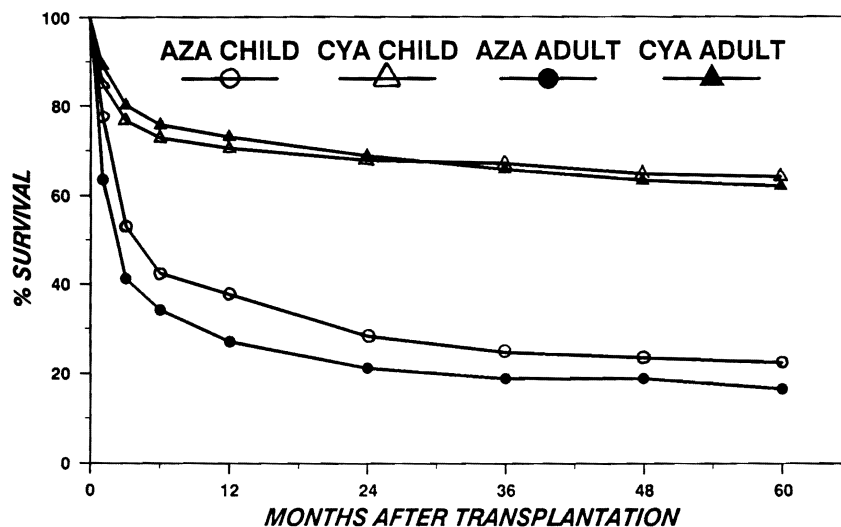
**TABLE 4.**

Renal Transplant Recipients Treated Before  
31 March 1964, Surviving in September 1989\*

|   | No. of<br>Patients | Original<br>Graft |
|---|--------------------|-------------------|
| University of Colorado                  | 14                 | 10                |
| Medical College of<br>Virginia          | 3                  | 3                 |
| University of<br>Minnesota              | 2                  | 2                 |
| Necker Hospital (Paris)                 | 1                  | 0                 |
| Peter Bent Brigham<br>Hospital (Boston) | 1                  | 1                 |
| Western General<br>Hospital (Edinburgh) | 1                  | 0                 |
| Cleveland Clinic                        | 1                  | 1                 |
| Total                                   | 23                 | 17                |

\*Data presented at the Sixth Capri Conference of Uremia. From Starzl TE, Schroter GPJ, Hartmann NJ, et al: Long term (25 years) survival after renal homotransplantation—The world experience. *Transplant Proc* 1990, (in press).





**FIG 46.**

Patient survival rates (life table method) for children (patients less than 18 years of age when they received their primary liver graft) and adults (patients 18 years of age or older when they received their primary liver graft). Eighty-five patients less than 18 years of age were treated with azathioprine (AZA) and steroids, and 438 were treated with cyclosporine (CYA) and steroids. Eighty-five patients 18 years of age or older were treated with azathioprine and steroids, and 1,031 were treated with cyclosporine and steroids.

Borel and associates of the phenomenal immunosuppressive qualities of cyclosporine,<sup>279</sup> with the initial clinical trials of this agent for cadaveric renal transplantation by Calne and co-workers,<sup>280, 281</sup> and with the systematic combination of cyclosporine with steroids and other immunosuppressive measures.<sup>282, 283</sup> Although cyclosporine and steroids are the baseline drugs, azathioprine is often used as a third maintenance agent to reduce the required dose of cyclosporine,<sup>284-289</sup> or it has been used in some cases to replace cyclosporine altogether after a few months or longer. Antilymphocyte globulin preparations,<sup>284</sup> including the monoclonal antibody OKT3,<sup>290-293</sup> have been given prophylactically, later in the postoperative period for the specific indication of rejection, because nephrotoxicity of cyclosporine necessitated its use in low doses, or both.

#### ***Cyclosporine and Its Limitations***

Cyclosporine has been the single most important factor in making liver transplantation a practical way of treating hepatic disease (see Fig 46). However, the drug's principal side effect of nephrotoxicity<sup>280, 281, 294</sup> puts a cap on its permissible dosage. Even with the

multiple-drug regimens mentioned in the preceding section, rejection has remained a common reason for early or late graft losses.<sup>67, 113</sup> Furthermore, it has not been possible to completely eliminate nephrotoxicity by using smaller doses of cyclosporine in drug cocktails. In human recipients of livers<sup>294-299</sup> and hearts,<sup>300-302</sup> evidence of renal dysfunction has included azotemia, hyperkalemia, and hypertension. Because the morphologic changes in the kidneys of these patients may not be reversible,<sup>297, 302-304</sup> the extent of the eventual liability of either short- or long-term cyclosporine therapy has yet to be determined.

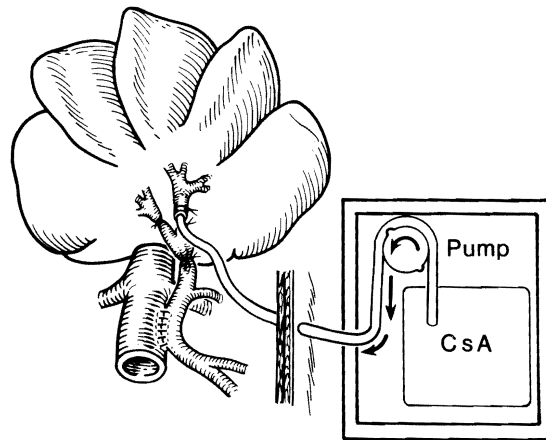
When cyclosporine was first used clinically in 1978 through 1981, assays were not available to monitor blood or plasma levels. Renal function was used to guide dosage, the objective being to give cyclosporine to the limit imposed by its nephrotoxicity.<sup>298, 305, 306</sup> There is much to be said for this approach even today. However, there is a tendency to guide cyclosporine doses by frequent measurements of blood or plasma trough concentrations with radioimmunoassay (RIA),<sup>307</sup> high-performance liquid chromatography (HPLC),<sup>308</sup> or fluorescence polarization immunoassay (FPIA).<sup>309</sup> A trough whole blood cyclosporine concentration of 250 to 450 ng/mL (HPLC), 800 to 1,200 ng/mL (RIA), or 1,000 to 1,600 ng/mL (FPIA) is normally considered to be therapeutic.<sup>309</sup> However, these so-called normal concentrations vary greatly from center to center. In addition, therapeutic concentrations for cyclosporine are dependent on the other immunosuppressive drugs used and may decrease with time after transplantation.<sup>310, 311</sup> Although some patients maintained at "therapeutic concentrations" have exhibited cyclosporine toxicity, others above the "therapeutic concentration" may not manifest any toxic symptoms at all. The maintenance of stable cyclosporine concentrations in liver transplant patients is more difficult than in recipients of other organs.<sup>312</sup> The changing quality of graft function postoperatively,<sup>313</sup> biliary duct obstruction or the presence or absence of T-tube drainage,<sup>314</sup> bile fistulas,<sup>315</sup> and numerous other factors common in or specific to liver transplant patients<sup>316</sup> make cyclosporine monitoring even more important than it is for kidney and heart transplant recipients,<sup>317</sup> providing reliable in-center standards are established.

#### *Cyclosporine and Liver Regeneration*

The ability of the liver to regenerate after being injured is an important consideration in any kind of major hepatic operation, but especially after liver transplantation where recovery from ischemic injury or from rejection is required in most cases. In addition, many chemotherapeutic agents inhibit regeneration, including doxorubicin (Adriamycin),<sup>318, 319</sup> which might be given to patients undergoing liver replacement for hepatic malignancies under cyclosporine immunosuppression. Consequently, it was important to know what ef-

fect cyclosporine has on regeneration. Earlier studies in rats showed that cyclosporine actually enhances the regeneration response after partial hepatectomy,<sup>320</sup> an unexplained effect that has been confirmed by other workers.<sup>321, 322</sup> The mechanisms of this seeming hepatotrophic effect will be important to determine for another reason, not only for cyclosporine but for other drugs. It is now known that the transplanted liver promptly goes through a period of volume adjustment, shrinking or enlarging to conform to an appropriate size for the particular recipient.<sup>323, 324</sup> It may be speculated from non-transplant experiments<sup>325</sup> that control of liver size is hormonal, with the most dominant factor being endogenous insulin. Interference or distortion of the hepatocellular growth control that is responsible would have practical implications.

The demonstration that cyclosporine enhances regeneration has prompted further experiments to elucidate its hepatotrophic properties. The model has been the dog submitted to end-to-side portacaval shunt (Fig 47).<sup>3</sup> The livers in animals with Eck fistula undergo acute atrophy and organelle disorganization within 4 days. The most specific organelle change caused by Eck fistula is disruption of the rough endoplasmic reticulum with depletion of its ribosomes.<sup>3, 325</sup> At the same time, the rate of hepatocyte mitoses per 1,000 hepatocytes increases from 1.5 to 4.5.<sup>3</sup>



**FIG 47.**

The use of an Eck fistula (portacaval shunt) model for the study of drugs such as cyclosporine (CsA). The model, in effect, splits the liver into two fragments that differ only by what is infused into the tied-off left portal vein branch. Each experiment serves as its own control, since the directly treated (*left lobar*) and control hepatocytes that are exposed to recirculate a drug (*right lobar*) are present in the same liver. (Redrawn from Starzl TE, Porter KA, Watanabe K, et al: *Lancet* 1976; 1:821–825.

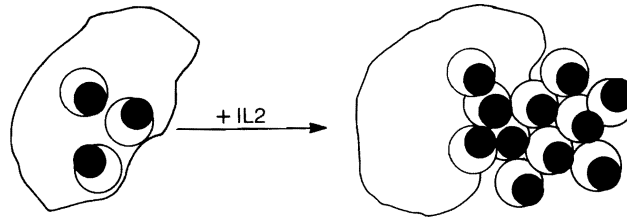
If insulin is perfused into the tied-off left portal vein (see Fig 47), the atrophy is prevented in the liver normally supplied by this branch, the organelle damage is prevented, and the rate of hepatocyte mitoses triples or quadruples. The contralateral hepatic lobes in these dogs are not affected by the insulin infusions, meaning that the insulin largely is consumed or inactivated with the first trans-hepatic passage.<sup>325</sup>

This same experiment has been performed with infusion of cyclosporine instead of insulin into the left portal vein.<sup>326</sup> The cyclosporine in appropriate doses prevents hepatocyte atrophy completely and increases proliferation slightly on the side of infusion. In contrast to insulin, the cyclosporine effect is almost as pronounced in the contralateral (right) liver lobes as in the infused ones. The fact that the cyclosporine hepatotrophic effect is not removed on first passage through the liver is of considerable interest, particularly since the liver is thought to be responsible for more than 90% of the degradation of this drug. A predominantly first passage removed of its hepatotrophic effect might have implied that cyclosporine is a liver-specific drug in other biologic actions as well, not excluding immunosuppression. The Eck fistula model with selective portal branch infusion may be a useful experimental device to study the effects of other orally administered drugs on the liver and to see how the liver alters these agents as they are picked up from the splanchnic venous bed during intestinal absorption and brought to the liver.

#### *A NEW DRUG: FK 506*

Until recently, only four drugs had been demonstrated to prolong liver graft survival in large animals: (1) azathioprine,<sup>327</sup> (2) antilymphocyte serum and its globulin derivative (ALG),<sup>276</sup> (3) cyclosporine,<sup>328</sup> and (4) the cyclosporine analogue Nva<sup>2</sup>-cyclosporine.<sup>329</sup> Recently, the efficacy of a new agent, FK 506, was demonstrated after canine liver transplantation.<sup>330, 331</sup> This agent might permit refinements of clinical immunosuppression. FK 506 was discovered in Japan less than 5 years ago and reported in the literature for the first time in 1987.<sup>330, 332-335</sup> A reasonably clear picture of the conditions that will permit the most effective and safest use of FK 506 has emerged from these studies. The practicality of combining FK with other conventional agents was shown with canine kidney and liver transplantation,<sup>331</sup> in which subtherapeutic doses of FK, cyclosporine, and steroids provided as good results as have ever been reported in dogs with any drug regimen.

The concept of drug synergism for immunosuppression is an old one<sup>273</sup> but difficult to prove until recently. Now, the interaction of drugs can be studied with great precision by measuring their effect on mixed lymphocyte culture systems.<sup>336, 337</sup> These techniques have

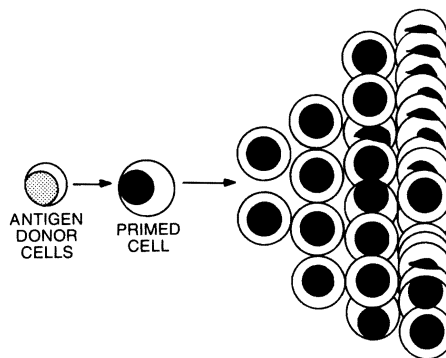


**FIG 48.**

Propagation of activated lymphocytes from human biopsy specimens with interleukin (*IL2*). (From Starzl TE: *Transplant Proc* 1988; 20[suppl 3]:356–360. Used by permission.)

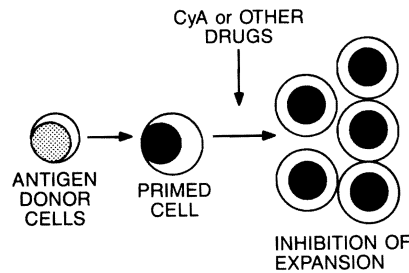
made it possible in tissue culture experiments to dissect the mechanisms of drug action as these affected lymphocyte populations, to study the intrinsic cytotoxicity of the agents on cell cultures, and to measure in highly quantifiable test systems the interactions (including synergism) of different drugs. It has been possible with a few days of effort to acquire information that previously was completely inaccessible or that required years to accumulate.

Zeevi and associates,<sup>337, 338</sup> Fung and colleagues,<sup>339</sup> and Duquesnoy and co-workers<sup>340</sup> in Pittsburgh have referred to these techniques as minitransplant models. From biopsy specimens of hearts and livers, they obtained cultures of primed lymphocytes that had been exposed to donor-specific antigen by virtue of transplantation (Fig 48). When donor spleen, which is saved at the time of organ har-



**FIG 49.**

Lymphocyte culture technique in which human lymphocytes obtained from biopsy specimens are cultured and exposed to donor cells. Clonal expansion results. (From Starzl TE: *Transplant Proc* 1988; 20[suppl 3]:356–360. Used by permission.)

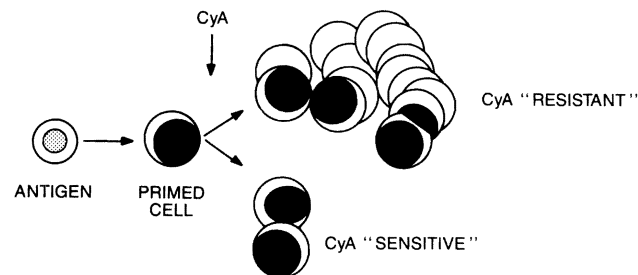


**FIG 50.**

Prevention or inhibition of clonal expansion in primed human lymphocyte cultures by addition of cyclosporine (CyA) or other drugs. (From Starzl TE: *Transplant Proc* 1988; 20[suppl 3]:356–360. Used by permission.)

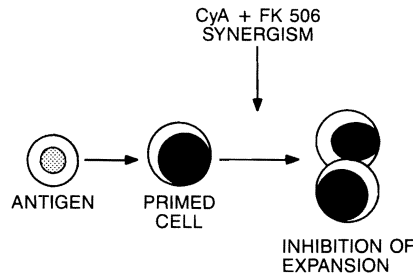
vest and preserved, is added to the recipient lymphocyte culture, the “primed” recipient lymphocytes proliferate (cell expansion) with very little delay (Fig 49). The mechanisms of the expansion can be studied qualitatively and quantitatively by collecting IL-2 or other lymphokines from the culture medium and adding them to IL-2-dependent cells. The proliferation or other response characteristics of these IL-2-dependent cells provide an end point for a biologic assay.

The ability of cyclosporine or other drugs to prevent this expansion of a human lymphocyte population is illustrated in Figure 50. In the liver or heart biopsy specimens of patients undergoing severe or even intractable rejection, clones of cyclosporine-resistant lymphocytes have been found side by side with sensitive clones (Fig 51).<sup>341</sup> In such cases, FK 506 used alone or added to cyclosporine can eliminate the rogue clones (Fig 52).<sup>342</sup> Cyclosporine, azathioprine,



**FIG 51.**

Development of cyclosporine (CyA)–resistant clones in liver or heart biopsy specimens that were undergoing clinical rejection. (From Starzl TE: *Transplant Proc* 1988; 20[suppl 3]:356–360. Used by permission.)



**FIG 52.**

Disappearance of "rogue" clones by the addition of the experimental drug FK 506 and cyclosporine (CyA). (From Starzl TE: *Transplant Proc* 1988; 20[suppl 3]:356–360. Used by permission.)

and FK 506 all are synergistic with each other with in vitro models.<sup>342, 343</sup>

In vivo synergism of FK 506 and cyclosporine has been demonstrated equally clearly with heterotopic heart transplantation in rats.<sup>344</sup> The synergism of FK 506 and cyclosporine is of special interest, since the two drugs have similar, if not identical, actions.<sup>334, 336, 337</sup>

FK 506 is remarkably nontoxic at therapeutic dose ranges in rats.<sup>335, 344, 345</sup> It can cause convulsive vomiting and lethal emaciation in dogs.<sup>330, 346–348</sup> Widespread arteritis was described in the organs of dogs,<sup>347, 348</sup> but in subsequent studies, these lesions were found in untreated control animals as well as in those given cyclosporine, steroids, or both.<sup>331</sup> Although one group has described alarming side effects of FK 506 in baboons,<sup>349</sup> further studies have been reassuring.<sup>331, 350</sup> In appropriate doses, the drug use alone in outbred baboon recipients has allowed nearly uniform survival of kidney homografts with minimal toxic side effects.<sup>350</sup>

Clinical trials with FK 506 recently were started in Pittsburgh, and the first dose was administered to a human on 28 February 1989. The patient is a 28-year-old woman who had been given three liver grafts over a period of 3 years. In addition to losing the first two livers to chronic rejection (Table 5), the recipient had developed renal failure to which cyclosporine nephrotoxicity was thought to have contributed. After FK 506 was started, rejection of the third liver graft was promptly controlled by histopathologic criteria (Table 6), with concomitant improvement of the liver chemistries (Table 7). However, her renal failure was not improved, and on March 27, 1989, cadaveric renal transplantation was carried out with immediate and sustained good renal function (Table 8). In this and all subsequent cases treated chronically, cyclosporine was eventually discontinued.

**TABLE 5.**

First Eight Liver Allograft Recipients Receiving FK 506

| Patient No. | I.D. | Age (yr) | Weight (kg) | FK 506 Start | OLT <sup>x</sup> No. | Date     | Cause of Liver Failure           |
|-------------|------|----------|-------------|--------------|----------------------|----------|----------------------------------|
| 1           |      | 28       | 50          |              | 0                    |          | Cryptogenic cirrhosis            |
|             |      |          |             |              | 1                    | 7/2/85   | Chronic rejection                |
|             |      |          |             |              | 2                    | 12/28/87 | Chronic rejection                |
|             |      |          |             | 2/28/89      | 3                    | 6/29/88  |                                  |
| 2           |      | 38       | 53          |              | 0                    |          | Sclerosing cholangitis           |
|             |      |          |             |              | 1                    | 11/9/83  | Primary nonfunction              |
|             |      |          |             |              | 2                    | 11/14/83 | Chronic rejection                |
|             |      |          |             |              | 3                    | 12/6/85  | Hepatic artery thrombosis        |
|             |      |          |             |              | 4                    | 2/16/86  | Chronic rejection                |
|             |      |          |             | 3/25/89      | 5                    | 1/1/89   | Hepatic artery thrombosis (late) |
|             |      |          |             |              | 6                    | 7/2/89   |                                  |
| 3           |      | 30       | 55          |              | 0                    |          | Autoimmune cirrhosis             |
|             |      |          |             |              | 1                    | 6/26/84  | Chronic rejection                |
|             |      |          |             | 4/4/89*      | 2                    | 11/18/87 |                                  |
| 4           |      | 43       | 52          |              | 0                    |          | Polycystic liver and kidney      |
|             |      |          |             | 4/8/89       | 1                    | 11/21/88 |                                  |
| 5           |      | 42       | 65          |              | 0                    |          | Cryptogenic cirrhosis            |
|             |      |          |             | 6/2/89       | 1                    | 4/30/89  |                                  |
| 6           |      | 38       | 49          |              | 0                    |          | Cryptogenic cirrhosis            |
|             |      |          |             | 6/29/89      | 1                    | 12/2/82  |                                  |
| 7           |      | 47       | 100         |              | 0                    |          | Sclerosing cholangitis           |
|             |      |          |             | 7/1/89       | 1                    | 6/15/86  |                                  |
| 8           |      | 18       | 63          |              | 0                    |          | Cryptogenic cirrhosis            |
|             |      |          |             |              | 1                    | 5/13/86  | Chronic rejection                |
|             |      |          |             | 7/8/89       | 2                    | 7/18/86  |                                  |

\*Because the allocated supply of IV FK 506 had been depleted, FK 506 was stopped on 8/5/89 (day 124), and cyclosporine was resumed.

When FK 506 and cyclosporine were used together, cyclosporine blood levels tended to rise with consequent aggravation of cyclosporine nephrotoxicity. The cadaveric kidney graft of patient no. 1, which has never been exposed to any baseline drug except for FK 506, has had no evidence of nephrotoxicity.

The same improvement in liver function has been noted in every patient except one (patient no. 4), whose initial diagnosis of rejection 4.5 months after combined liver and kidney transplantation proved to be incorrect. Within a few days, it was realized that this patient had fulminant hepatic failure due to B virus hepatitis, and the FK 506 was stopped. Despite retransplantation, the patient died.

The remarkable effectiveness of FK 506 in patients for whom all previous therapy had failed, as well as the seeming lack of toxicity in these patients, has been noteworthy. From the preliminary observa-



**TABLE 6.**

Description of Liver Biopsy Results Before and After FK 506\*

| Patient I.D<br>No. | Day | Cellular Infiltrate | Ductal Damage | Duct Loss | Fibrosis |
|--------------------|-----|---------------------|---------------|-----------|----------|
| 1                  | 0   | 1+                  | 1+            | 0         | 0        |
|                    | 15  | 0                   | 0             | 0         | 0        |
|                    | 69  | 0                   | 0             | 0         | 0        |
| 2                  | 0   | 1+                  | 1+            | 0         | 0        |
|                    | 12  | 1+                  | 0             | 0         | 0        |
|                    | 34  | 0                   | 0             | 0         | 0        |
|                    | 94  | 0                   | 0             | 0         | 0        |
| 3                  | 0   | 2+                  | 1+            | 0         | 0        |
|                    | 14  | 0                   | 0             | 0         | 0        |
|                    | 31  | 0                   | 0             | 0         | 0        |
| 4 <sup>†</sup>     | 0   | 1+                  | 1+            | 0         | 0        |
| 5                  | 0   | 3+                  | 2+            | 0         | 2+       |
|                    | 14  | 1+                  | 1+            | 0         | 2+       |
|                    | 51  | 0                   | 0             | 1+        | 1+       |
| 6                  | 0   | 1+                  | 3+            | 0         | 0        |
|                    | 12  | +/-                 | 2+            | 0         | 1+       |
| 7                  | 0   | 1+                  | 1+            | 1+        | 0        |
|                    | 18  | 0                   | 1+            | 0         | 0        |
| 8                  | 0   | 2                   | 2+            | 1+        | 1+       |
|                    | 16  | 1                   | 1+            | 1+        | 1+       |

\*Scale from 0-3+, with 0 being no injury and 3+ being extensive injury.

†Initial diagnosis of cellular rejection was incorrect; special staining for hepatitis B core and surface antigen was positive. Patient no. 4 progressed to fulminant hepatic failure, although FK 506 was stopped.

**TABLE 7.**

Response of Liver Function Tests to FK 506\*

| Patient I.D.<br>No. | Day | TBIL<br>(mg/dL) | SGOT<br>(IU/L) | SGPT<br>(IU/L) | Alkaline Phosphate<br>(IU/L) | GGTP<br>(IU/L) |
|---------------------|-----|-----------------|----------------|----------------|------------------------------|----------------|
| 1                   | 0   | 0.6             | 49             | 47             | 160                          | 71             |
|                     | 7   | 0.6             | 24             | 24             | 145                          | 45             |
|                     | 14  | 0.5             | 31             | 36             | 131                          | 42             |
|                     | 28  | 0.2             | 11             | 10             | 75                           | 24             |
|                     | 56  | 0.2             | 17             | 17             | 111                          | 37             |
|                     | 143 | 0.2             | 20             | 19             | 94                           | 22             |
|                     | 156 | 0.3             | 29             | 27             | 90                           | 17             |
| 2                   | 0   | 1.3             | 109            | 142            | 345                          | 167            |
|                     | 7   | 0.7             | 46             | 109            | 277                          | 172            |

(Continued.)

**TABLE 7 (cont.).**

| Patient I.D.<br>No. | Day  | TBIL<br>(mg/dL) | SGOT<br>(IU/L) | SGPT<br>(IU/L) | Alkaline Phosphate<br>(IU/L) | GGTP<br>(IU/L) |
|---------------------|------|-----------------|----------------|----------------|------------------------------|----------------|
|                     | 14   | 0.8             | 50             | 77             | 214                          | 146            |
|                     | 28   | 0.5             | 29             | 30             | 145                          | 83             |
|                     | 56   | 0.4             | 29             | 36             | 70                           | 53             |
|                     | 109† | 3.6             | 33             | 105            | 514                          | 1,091          |
|                     | 116† | 1.9             | 80             | 171            | 858                          | 1,267          |
|                     | 123† | 1.2             | 35             | 29             | 311                          | 265            |
|                     | 130† | 0.7             | 28             | 31             | 352                          | 256            |
|                     | 143† | 0.9             | 46             | 71             | 394                          | 284            |
| 3                   | 0    | 0.6             | 32             | 32             | 121                          | 82             |
|                     | 7    | 0.5             | 41             | 31             | 117                          | 88             |
|                     | 14   | 0.4             | 26             | 24             | 96                           | 76             |
|                     | 28   | 0.2             | 11             | 10             | 99                           | 64             |
|                     | 56   | 0.6             | 26             | 9              | 81                           | 33             |
|                     | 102‡ | 1.7             | 53             | 20             | 192                          | 174            |
|                     | 124‡ | 1.9             | 56             | 49             | 297                          | 370            |
| 4                   | 0    | 2.3             | 287            | 312            | 144                          | 108            |
| 5                   | 0    | 20.5            | 63             | 79             | 417                          | 437            |
|                     | 7    | 13.6            | 60             | 72             | 386                          | 498            |
|                     | 14   | 9.3             | 47             | 60             | 257                          | 361            |
|                     | 28   | 3.8             | 38             | 61             | 305                          | 350            |
|                     | 56   | 1.9             | 31             | 38             | 229                          | 215            |
|                     | 63   | 1.5             | 9              | 39             | 194                          | 185            |
|                     | 69   | 1.5             | 13             | 38             | 173                          | 112            |
| 6                   | 0    | 2.5             | 339            | 634            | 348                          | 2,756          |
|                     | 7    | 0.7             | 33             | 107            | 283                          | 1,536          |
|                     | 14   | 0.5             | 46             | 95             | 557                          | 2,742          |
|                     | 28   | 0.5             | 93             | 114            | 409                          | 2,457          |
|                     | 46   | 0.5             | 52             | 75             | 246                          | N.D.           |
| 7                   | 0    | 2.1             | 198            | 550            | 304                          | 1,351          |
|                     | 7    | 1.6             | 40             | 236            | 250                          | 876            |
|                     | 14   | 1.2             | 135            | 600            | 523                          | 1,287          |
|                     | 28   | 0.7             | 94             | 324            | 501                          | 1,593          |
|                     | 41   | 1.8             | 114            | 408            | 555                          | 1,306          |
| 8                   | 0    | 3.7             | 713            | 609            | 268                          | 1,348          |
|                     | 7    | 1.2             | 318            | 284            | 176                          | 751            |
|                     | 14   | 1.1             | 175            | 128            | 119                          | 433            |
|                     | 28   | 0.8             | 84             | 90             | 106                          | 254            |
|                     | 38   | 0.5             | 74             | 86             | 142                          | 192            |

\*TBIL = total bilirubin; SGOT = serum glutamic oxaloacetic transaminase; GGTP = gamma glutamy/ transpeptidase.

†New liver allograft (see text).

‡On IV hyperalimentation (see text); FK 506 stopped on day 124.

**TABLE 8.**

Renal Function, Cyclosporine, and FK 506

| Patient I.D.<br>No. | Day  | Cyclosporine Oral Dose Level |       | FK 506 Oral Dose Level |       | BUN*<br>(mg/dL) | Creatinine<br>(mg/dL) |
|---------------------|------|------------------------------|-------|------------------------|-------|-----------------|-----------------------|
|                     |      | mg/d                         | ng/mL | mg/d                   | ng/mL |                 |                       |
| 1                   | 0    | 900                          | 685   | 0                      | 0     | 50              | 2.5                   |
|                     | 7    | 150                          | 737   | 18                     | 0.4   | 98              | 4.1                   |
|                     | 14   | 0                            | 281   | 18                     | 0.7   | 81              | 4.1                   |
|                     | 28   | 0                            | 0     | 18                     | 0.3   | 51              | 2.8                   |
|                     | 56   | 0                            | 0     | 18                     | 0.5   | 38              | 1.4                   |
|                     | 143  | 0                            | 0     | 18                     | 0.4   | 29              | 1.4                   |
|                     | 156  | 0                            | 0     | 18                     | 0.4   | 23              | 1.3                   |
| 2                   | 0    | 1,600                        | 255   | 0                      | 0     | 37              | 1.7                   |
|                     | 7    | 100                          | 810   | 18                     | 0.4   | 50              | 2.0                   |
|                     | 14   | 0                            | 0     | 18                     | 0.7   | 61              | 2.5                   |
|                     | 28   | 0                            | 0     | 18                     | 0.2   | 45              | 2.4                   |
|                     | 56   | 0                            | 0     | 9                      | 0.3   | 38              | 2.4                   |
|                     | 109  | 0                            | 0     | 6 †                    | 1.1   | 111             | 3.6                   |
|                     | 130  | 0                            | 0     | 3 †                    | 0.4   | 63              | 1.7                   |
|                     | 143  | 0                            | 0     | 9 †                    | 0.9   | 65              | 2.6                   |
| 3                   | 0    | 150                          | 214   | 0                      | 0     | 84              | 5.4                   |
|                     | 7    | 0                            | 0     | 18                     | 1.0   | 95              | 6.5                   |
|                     | 14   | 0                            | 0     | 18                     | 1.0   | 88              | 5.8                   |
|                     | 28   | 0                            | 0     | 12                     | 0.7   | 60              | 5.8                   |
|                     | 124† | 50                           | < 50  | 0                      | 0.1   | 34              | 3.2                   |
| 4                   | 0    | 800                          | 1,621 | 0                      | 0     | 75              | 2.4                   |
| 5                   | 0    | 2,000                        | 967   | 0                      | 0     | 25              | 0.6                   |
|                     | 7    | 150                          | 1,373 | 20                     | 4.0   | 49              | 1.4                   |
|                     | 14   | 150                          | 148   | 18                     | 1.2   | 49              | 1.7                   |
|                     | 28   | 150                          | 190   | 20                     | 1.9   | 46              | 1.4                   |
|                     | 56   | 150                          | 324   | 20                     | 0.6   | 40              | 1.5                   |
|                     | 69   | 0                            | 0     | 20                     | 1.2   | 40              | 1.6                   |
| 6                   | 0    | 100                          | 154   | 0                      | 0     | 55              | 2.3                   |
|                     | 7    | 100                          | 160   | 16                     | 1.2   | 65              | 2.7                   |
|                     | 14   | 0                            | 76    | 16                     | 0.9   | 82              | 3.8                   |
|                     | 28   | 0                            | 0     | 16                     | N.A.  | 55              | 4.0                   |
|                     | 46   | 0                            | 0     | 16                     | 2.1   | 58              | 3.5                   |
| 7                   | 0    | 250                          | 482   | 0                      | 0     | 38              | 2.1                   |
|                     | 7    | 100                          | 615   | 30                     | N.A.  | 37              | 2.5                   |
|                     | 14   | 100                          | 292   | 30                     | 3.9   | 41              | 2.4                   |
|                     | 28   | 0                            | 0     | 30                     | 1.8   | 57              | 2.9                   |
|                     | 41   | 0                            | 0     | 30                     | 2.1   | 49              | 3.1                   |
| 8                   | 0    | 440                          | 845   | 0                      | 0     | 29              | 1.1                   |
|                     | 7    | 100                          | 444   | 18                     | 3.1   | 40              | 2.6                   |

(Continued.)

TABLE 8 (cont.).

| Patient I.D.<br>No. | Cyclosporine Oral Dose Level |      |       | FK 506 Oral Dose Level |       | BUN*<br>(mg/dL) | Creatinine<br>(mg/dL) |
|---------------------|------------------------------|------|-------|------------------------|-------|-----------------|-----------------------|
|                     | Day                          | mg/d | ng/mL | mg/d                   | ng/mL |                 |                       |
|                     | 14                           | 0    | 129   | 18                     | 3.0   | 40              | 3.6                   |
|                     | 28                           | 0    | 0     | 18                     | N.A.  | 66              | 3.1                   |
|                     | 38                           | 0    | 0     | 9.5                    | 3.9   | 29              | 2.9                   |

\*BUN = blood urea nitrogen.

†Following liver transplant (see text).

‡Value on hemodialysis.

tions made with FK 506 as a salvage drug, its efficacy and safety seem beyond question even at this early stage. A trial of FK 506 as the primary drug in liver transplantation was started in August 1989.

A very important observation in the patients with FK 506 has been almost immediate relief from the severe hypertension from which each of the patients except patient no. 4 were suffering. The antihypertensive therapy was greatly reduced or stopped altogether in these patients.

At the annual meeting of the European Society of Organ Transplantation, which was convened on October 31, 1989, a complete report of FK 506 was given, including exposition of the Pittsburgh clinical trials. The FK 506 has been synthesized, and its binding site has been identified by Dr. Stuart Schreiber of Harvard University. This binding site, which has been called *Fujiphilin*, is different than the cyclophilin binding site for cyclosporine.

In the meanwhile, an additional drug with a very similar chemical structure has been isolated by workers of the Ayerst Pharmaceutical Corporation from the fungus *Streptomyces hygroscopicus*<sup>351</sup> and called rapamycin. This drug has been described as having powerful immunosuppressive qualities in rodents and dogs.<sup>352</sup> It seems clear that the FK 506 is the forerunner of a new and extremely interesting class of drugs that may have a potency and safety profile good enough to make them competitive with or possibly superior to cyclosporine.

## DIFFERENTIAL DIAGNOSIS OF GRAFT DYSFUNCTION

When a liver graft fails intraoperatively, nonimmunologic factors are the primary suspects, even though there may be exceptions, as described in the previous section. The frame of reference quickly changes thereafter. Immunologic rejection as an explanation for later graft dysfunction becomes increasingly probable with each passing day after transplantation, particularly if the new liver seemed to be satisfactory at the outset. Nevertheless, nonimmunologic explanations for delayed graft failure or dysfunction must be systematically ruled out. During the first several postoperative months, the diagnostic possibilities include suboptimal revascularization, as already discussed; defects in bile duct reconstruction causing obstruction or fistula; opportunistic viral infection with cytomegalovirus (CMV),<sup>353, 354</sup> herpes simplex virus (HSV) or viruses,<sup>354</sup> Epstein-Barr virus (EBV),<sup>355</sup> or adenovirus (ADV)<sup>356, 357</sup>; infection by a variety of bacterial or fungal pathogens<sup>358</sup>; toxicity from hyperalimentation or sepsis<sup>144, 145</sup>; and hepatotoxicity of the drugs used to prevent rejection<sup>359, 360</sup> or for other purposes.<sup>361</sup> Graft dysfunction occurring at a somewhat later time can be caused by recurrence of the disease that destroyed the native liver, infection of the transplant by one of the hepatitic viruses, defects of bile duct reconstruction, or chronic rejection. Each general cause of graft dysfunction except those already covered will be expanded on in the following sections.

### *REJECTION AND TOLERANCE INDUCTION*

Like other immune responses, rejection can be separated into three distinct but overlapping phases: (1) recognition of the antigen (induction), (2) development of response capable of neutralizing the antigen (effector), and (3) regulatory mechanisms that restore homeostasis to the organism.<sup>362, 363</sup> It is likely that there are several different inductor, effector, and regulatory pathways involved in each phase.<sup>362, 363</sup> Clinically, effector mechanism receive the most attention, since recognition and attempts to control this process are the

mainstays of recipient management. Inductive and regulatory pathways remain largely in the realm of experimental transplantation and at present seem to be the least understood.<sup>364-366</sup>

Aside from its resistance to humoral rejection (see earlier section), the liver displays some special properties as a solid organ allograft in both animals and humans and therefore serves as an especially good model to study each phase of the rejection response. Shortly after the initiation of human liver transplantation, Cordier and associates<sup>367</sup> as well as others<sup>368</sup> discovered that liver allografts in pigs do not follow the normal laws of transplantation. They found that porcine hepatic grafts experienced prolonged survival with little or no immunosuppression. Calne and co-workers<sup>369-371</sup> demonstrated that along with the immunologic "privileged" status, porcine liver allografts also induced a state of hyporesponsiveness to other tissues from the same donor. In contrast, no spontaneous long-term liver allograft survival was seen in the dog, baboon, rhesus monkey, or humans, all of whom required immunosuppressive therapy to maintain graft viability.<sup>372</sup> Later, Zimmerman and colleagues<sup>373</sup> and Kamada and others<sup>273, 374-376</sup> demonstrated that inbred strains of rats experienced a phenomenon similar to that seen in pigs. Since then, the rat has served as an invaluable animal model for the study of liver transplantation.<sup>213, 376</sup> The resistance of the liver allograft to hyperacute rejection has already been discussed.

#### *Tolerance Induction and Immunosuppression Induced by Rat Liver Transplantation*

As mentioned previously, liver allografts are permanently accepted without immunosuppression between certain strains of rats (e.g., DA to PVG), whereas in others, the liver is acutely rejected.<sup>213, 376, 377</sup> The class II MHC antigens appear to be the most influential in determining the rejector status of the strain combinations.<sup>213, 378, 379</sup> However, even across full RT1 haplotype mismatches, liver allografts are tolerated in these nonrejector combinations, whereas other organs (e.g., skin, heart, and kidney) are acutely rejected.<sup>374-376</sup> The liver grafts also induce a state of donor-specific unresponsiveness in the recipient that permits subsequent transplantation of the skin, heart, or kidney grafts.<sup>213, 375, 376</sup> Liver grafts performed on the same day as the kidney or heart graft can prevent subsequent rejection of either of these extrahepatic organs.<sup>374</sup> However, a period of at least 5 days is required between the liver and skin grafts to achieve any acceptance.<sup>374</sup> Liver grafts are even able to reverse cellular rejection in cardiac grafts transplanted 5 to 6 days before the liver.<sup>213, 376</sup> This potent tolerance-enhancing effect is also capable of reversing a pre-sensitized state (i.e., removing circulating allogeneic antibodies and memory cells).<sup>213, 376</sup> However, when liver grafts are transplanted to

presensitized recipients, the acceptance rate falls from 95% to 50% (see the discussion of antibody-mediated rejection).<sup>213, 376</sup>

This tolerance-enhancing and immunosuppressant effect seems to be dependent on removal of the recipient liver.<sup>380, 381</sup> Auxiliary grafts are almost invariably rejected, and the recipient becomes sensitized as a result.<sup>380, 381</sup> The reasons for these observations are largely unknown; however, the immunogenicity of the liver seems to reside largely in the nonparenchymal cell fraction. Sensitization rather than tolerance develops following infusion of unfractionated liver cell suspensions that contain both parenchymal and nonparenchymal elements.<sup>380</sup> Lautenschlager and others<sup>382, 383</sup> infused crude subfractions of liver-derived cells in an attempt to prime recipients for rejection of subsequent heart grafts. They found little immunogenicity associated with the fraction enriched in hepatocytes,<sup>382, 383</sup> whereas the Kupffer cell fraction, which may also have contained dendritic cells, was potentially immunogenic.

#### *Possible Mechanisms Underlying the Unique Properties of Liver Allografts in Rats*

Genetic control of the allogeneic immune response is the most obvious reason for the nonresponder status in rats, since the phenomenon described earlier occurs only between certain strain combinations. As might be expected, in rat liver transplantation the allogeneic response appears to be under the control of primarily the immune response gene (Ia or class II MHC), but minor polymorphic MHC loci may also influence the reaction.<sup>213, 376, 379, 384</sup> Although no unifying concept has been described to explain the peculiarities associated with rat liver transplantation, many of the effects observed are similar to those seen when attempts are made to regulate other immune responses. Kamada and Wight,<sup>374</sup> Zimmerman and colleagues,<sup>373</sup> and Houssin and associates<sup>214</sup> reported that rat liver allografts secrete soluble MHC antigens in the circulation where they bind to antigraft antibodies, rendering them nontoxic. However, it has been difficult to detect circulating immune complexes. Human liver allografts also secrete these MHC products,<sup>211</sup> and their binding to preformed antibodies is one mechanism whereby the liver is thought to be relatively resistant to the effects of preformed lymphocytotoxic antibodies.

Kamada and associates<sup>385</sup> have also shown that serum from liver graft-tolerant (LGT) rats can cause donor-specific enhancement of heart grafts, and the enhancing activity has been localized to the anti-Ia antibody subfraction.<sup>386</sup> Lymph fluid from LGT rats exhibits a similar effect but requires daily administration.<sup>213, 376</sup>

Although liver grafts are eventually tolerated between nonrejec-

tor strain combinations in rats, they undergo a histologically and biochemically documentable episode of acute cellular rejection.<sup>213, 376, 387</sup> This initial reaction is associated with inflammatory cell infiltration of the graft and a low transient elevation of anti-class I antibodies.<sup>213, 376, 388</sup> Thereafter, graft infiltrating cells subside, and the class I antibodies return to baseline.<sup>388</sup> Persistent high-titer anti-class II antibodies subsequently appear and may be partially responsible for maintenance of the graft.<sup>213, 376, 387, 388</sup> Immunophenotypic analysis of graft infiltrating cells during the transient rejection episode in nonrejector rats reveals a profile of cells quantitatively similar to that in rejector strain combinations.<sup>389, 390</sup> Qualitatively, however, the ratio of T cells to non-T cells and T-helper cells to T-suppressor/cytotoxic cells are increased over time in nonrejector combinations compared with the rejector strains.<sup>389, 390</sup> In addition, eventual hepatocyte necrosis with architectural collapse, which presumably is the result of the vascular insufficiency, never develops in grafts that are eventually tolerated (unpublished observations).

Adoptive transfer of thoracic duct lymphocytes of LGT rats has no effect in the immunologically crippled host.<sup>213, 376</sup> However, transfer of graft-infiltrating lymphocytes restores the alloreaction, suggesting that clonal deletion of donor-specific effector cells occurs within the liver graft.<sup>375, 376</sup> Despite the inability of the animal to reject the liver, in vitro, lymphocytes from LGT rats proliferate in response to donor lymphoid cell and generate CTLs.<sup>213, 376</sup> Also, in vivo localized graft-vs.-host (GVH) lymph node reactions remain intact. This phenomenon has been termed *split tolerance*.<sup>213, 376</sup> Splenic suppressor cells have also been identified.<sup>391</sup> Similar immunologic findings have been reported in nonrejector pig strain combinations<sup>392</sup> and in some human liver allograft recipients.<sup>393</sup>

The immunologic observations in LGT rats are similar to those seen in antibody enhancement studies. The antigen reactive cell opsinization (ARCO) hypothesis has been used to explain the relationship between delayed-type hypersensitivity responses, which are thought to be important in rejection, and antibody reactions.<sup>394</sup> This hypothesis incorporates a role for antigens and antibodies, suppressor cells, splenic sequestration, and clonal deletion of alloreactive cells in the liver, all of which are reportedly seen in LGT rats. The position of the liver in the circulation and the function of the intrahepatic reticuloendothelial system may be important in this regard. Several groups have reported prolonged survival of various allografts following portal venous inoculation of allogeneic cells.<sup>395, 396</sup> However, others have been unable to reproduce this phenomenon, and Starzl and colleagues have questioned the experimental basis and rationale of this approach.<sup>3</sup>



### *Inductive Pathways*

We will now return to consideration of rejection as an allogeneic immune response and its clinicopathologic manifestations. As was shown by Medawar,<sup>256, 397</sup> recipients reject foreign tissue allografts because of an immunologic reaction elicited by a genetic disparity between the donor and recipient, which demonstrates both specificity and memory. The response is largely T-cell dependent and is provoked by the cell surface glycoproteins encoded by the MHC complex on chromosome 6 in humans. Not only are these antigens the principal targets on the transplanted tissue, but they assist in the regulation of the recipient rejection response.<sup>398</sup> Other antigens of importance in the rejection response include the major ABO blood group system, minor MHC antigens, and possibly tissue-specific antigens.<sup>362, 363</sup>

Despite the observation that MHC antigens provoke strong rejection responses when they are part of an allograft, as isolated antigens, they are, in general, considered to be relatively weak immunogenes within species.<sup>362, 363, 399</sup> A strong *in vivo* cytotoxic T-lymphocyte response to these antigens requires not only the antigen but also a second signal, or costimulus, which is provided by a viable donor cell.<sup>399</sup>

Donor accessory, especially "dendritic" or passenger leukocytes are capable of presenting both the foreign MHC antigen and providing the second signal, or costimulus.<sup>399, 400</sup> The ability to respond *de novo* to alloantigens has been attributed to the diversity and cross-reactivity within the antigen and MHC restriction element sites on the T-cell receptor complex.<sup>401-404</sup> Clones that normally recognize self-antigen X (e.g., viruses) complexes can cross-react with alloantigens.<sup>401-404</sup> Alternatively, the donor MHC antigens may be processed by recipient antigen-presenting cells, similar to other types of foreign antigens.<sup>362, 363</sup>

The structures within the allografts that trigger the alloreaction have not been identified with certainty, nor is it known whether the inductive phase occurs within the graft, systemically, or both. Liver grafts offer a unique opportunity to study the sites of sensitization because of the strict structural anatomy of the organ. In a "normal" untransplanted liver, such as a donor liver prior to transplantation, there is strong expression of the major ABO blood group antigens on arterial venous and capillary endothelium and bile duct cells.<sup>405</sup> Hepatocytes do not express any of these antigens. The class I MHC antigens are expressed strongly on the bile ducts and somewhat more weakly on the sinusoidal cells and endothelial cells. Class I MHC antigens are barely detectable on hepatocytes. Class II MHC antigens (DR, DQ, and DP) are expressed only on capillary endothelium, sinusoidal cells and dendritic-shaped cells within the portal tri-

ads.<sup>238, 383, 405–413</sup> The presence of MHC antigens on the cell surface, however, is a dynamic process influenced by disease, drugs, inflammation, and circulating immune mediators and is altered after transplantation, which will be discussed later.

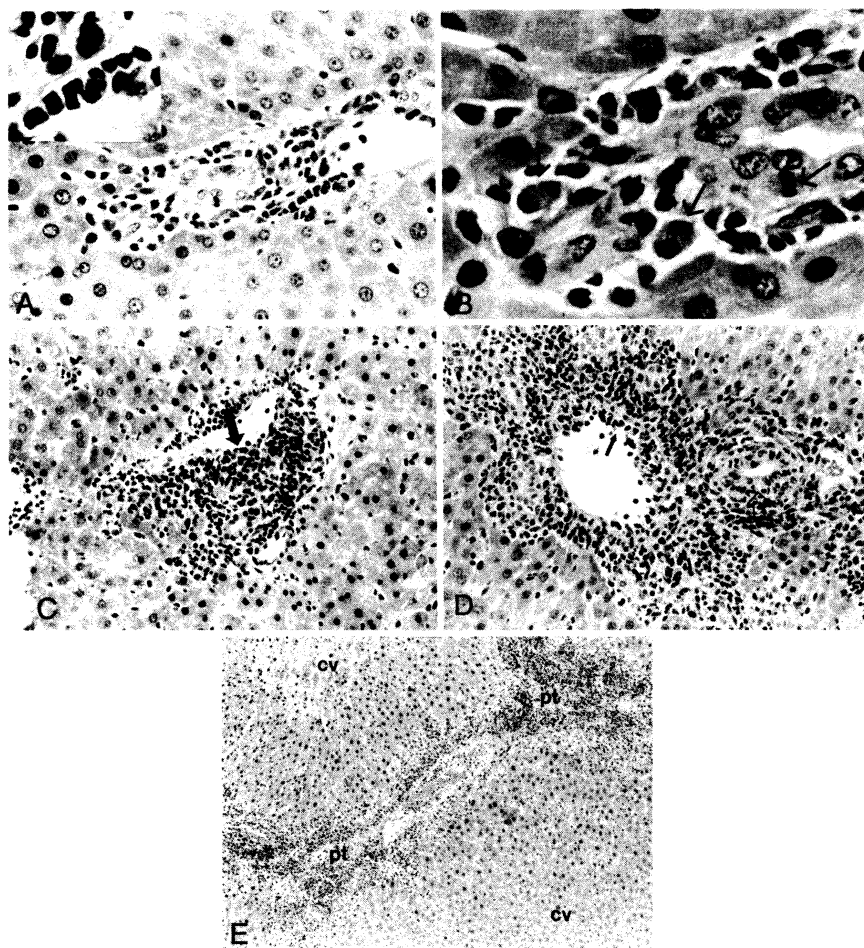
Until recently, little attention has been given to the possible role of dendritic cells (DCs) in liver allograft rejection.<sup>409, 414</sup> Dendritic cells have been shown to be the most potent stimulators of the mixed lymphocyte response and spontaneous DC-allogeneic lymphocyte clustering is observed within hours after the initiation of a mixed lymphocyte culture.<sup>400, 415</sup> In the liver, DCs are thought to be localized almost exclusively within the portal tracts,<sup>409, 414</sup> although more definitive work is needed in this area.

Daily histopathologic examination of rejector strain combination animal or some human liver allografts reveals what may be the morphologic correlate of the inductive phase of the immune response. Two to 3 days after graft implantation, mononuclear cells begin to sludge and cluster in the capillaries and interstitium of the portal tract. At this time, mitotic figures can easily be identified in these accumulating lymphoid cells (Fig 53), which suggests that at least some degree of sensitization occurs within the liver. Structures located at this initial site of accumulation and likely responsible for triggering the immune reaction include the donor DCs,<sup>409, 414</sup> capillary and lymphatic endothelia, and other connective tissue cells. Thereafter, infiltration and damage to target structures signal the beginning of the effector phase (see Fig 53).

### *Effector Pathways*

Several pathways have been implicated in the effector phase of the alloreaction: direct antibody and complement mediated damage, delayed-type hypersensitivity responses, cytotoxic T lympholysis, and antibody-dependent cell cytotoxicity mediated through killer cells.<sup>362, 363, 416–422</sup> All of these effector pathways are dependent on T lymphocytes.<sup>362, 363, 416–422</sup>

These pathways roughly correspond to clinical classification of rejection.<sup>362, 363</sup> Direct antibody and complement-mediated damage is largely responsible for triggering the cascade of events resulting in hyperacute rejection. Delayed-type hypersensitivity and allogeneic cytotoxic T lympholysis play principal roles in acute cellular rejection, and chronic rejection most likely represents a vascular directed attack by a combination of both cellular and humoral immunity. However, the present clinicopathologic classification of rejection into hyperacute, acute, and chronic rejection is not ideal, particularly with regard to the liver, and is probably in need of revision. Nevertheless, we will adhere to conventional terminology in the present review.



**FIG 53.**

Early histologic events in rejecting rat liver allograft (BN→DA). **A**, the early evidence of the alloresponse occurs at 2 days and consists of mononuclear cell sludging in the capillaries (*inset*) and interstitium of the portal tracts. **B**, closer examination of this population reveals mitotic figures (*arrows*), suggesting that some sensitization may occur within the graft. **C**, by 4 days, the infiltrate begins to tunnel beneath the portal vein endothelium (*arrow*); **D**, by 5 days, venous damage and infiltration into the ducts are noted (*arrows*). **E**, preterminal changes at 12 to 14 days include portal-portal linkage, centrilobular collapse, congestion and hemorrhage (*pt* = portal tract; *cv* = central vein). (From Demetris AJ, Qian S, Sun H, et al: *Am J Surg Pathol* [in press]. Used by permission.)

#### CLINICOPATHOLOGIC FEATURES OF REJECTION

##### *Acute Cellular Rejection*

The physiologic and morphologic features of cellular rejection were worked out long ago in experimental animals.<sup>8, 9, 18, 74, 81, 186, 327, 423</sup> Improvement in patient survival (and wide-

spread utilization of liver transplantation) has enabled an expansion of some of these basic observations.<sup>118, 119, 144, 145, 179, 424-435</sup> In human liver allograft recipients, acute cellular rejection is the most common and principal manifestation of the rejection reaction. Most episodes occur between 6 or 7 days and 6 weeks after transplant but may be seen as early as 2 or 3 days after the operation. Episodes occurring later than 2 months usually, but not invariably, are associated with decreased levels of immunosuppressive agents. The clinical signs of acute rejection include fever, lethargy, graft tenderness, leukocytosis, and a change in the color or quantity of bile.<sup>18, 66, 67, 74, 435-437</sup> Peripheral blood and graft eosinophilia<sup>438, 439</sup> and lymphocytosis<sup>440</sup> have also been associated with rejection, as have increased levels of serum neopterin,<sup>441</sup> soluble IL-2 receptors,<sup>442</sup> guanine,<sup>443</sup> amyloid A protein, and  $\beta_2$ -microglobulin,<sup>444</sup> but none of these alterations appears to be entirely specific. Serum bilirubin is a sensitive marker of dysfunction, and hepatic enzymes indicative of liver injury are frequently increased, but neither the absolute level nor the pattern of elevation is specific for rejection.<sup>18, 66, 67, 74, 435-437</sup> Confirmation of a clinical suspicion rejection is usually achieved by core needle biopsy evaluation.\*

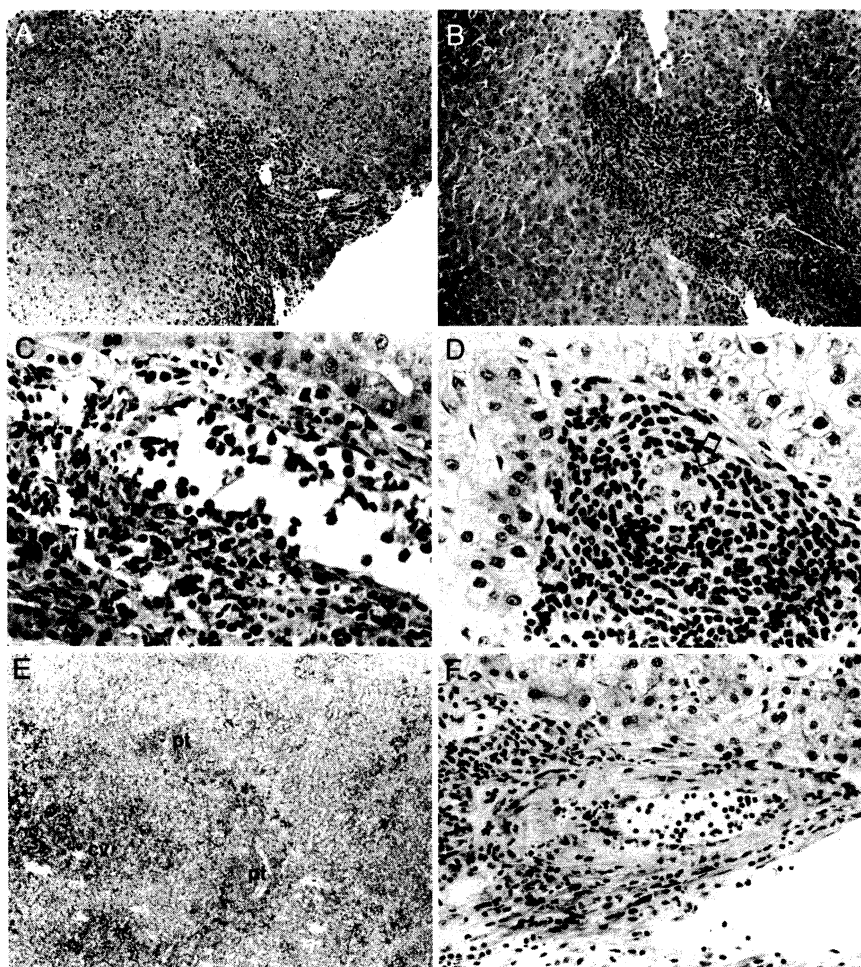
The histologic diagnosis of acute cellular rejection rests mostly on identification of a predominantly mononuclear portal tract inflammatory infiltrate, along with evidence of tissue damage (Fig 54).<sup>†</sup> It should be emphasized that portal inflammation alone may be due to many causes and therefore is not diagnostic of rejection. The initial accumulation of mononuclear cells occurs in the interstitium of the portal tracts. Tissue damage becomes manifest as the infiltrate extends into the walls of the portal vein and bile ducts, associated with reactive changes in the target cell populations (endothelium and bile ducts) such as hypertrophy and nuclear enlargement. Evidence of pyknosis and focal necrosis is also seen.

Cytologically the rejection infiltrate consists of an admixture of large blastic lymphocytes, smaller lymphocytes, plasma cells, macrophages, eosinophils, and neutrophils. Eosinophils may predominate in some cases during the early phases, simulating an allergic drug reaction.<sup>144, 145, 428, 438</sup> Immunophenotypic analysis of the rejection infiltrate demonstrates a preponderance of T cells with both CD4<sup>+</sup> or CD8<sup>+</sup> subsets; non-T cells, such as macrophages, monocytes, neutrophils, and B cells, are also present.<sup>238, 409, 411, 445, 446</sup>

Hepatic arteries within the portal tract are difficult to locate during an acute cellular rejection episode. Endothelial swelling and mural hypertrophy are the most common observation when the arteries are found. Necrotizing or neutrophilic arteritis (or both) is rarely

\*References 67, 118, 119, 144, 145, 179, 423-436.

†References 67, 118, 119, 144, 145, 179, 423-434.



**FIG 54.**

Histopathology of acute cellular rejection in humans. Events are almost identical to those seen in the rat (see Fig 53). **A**, mild acute cellular rejection. **B**, in moderate acute cellular rejection, the infiltrate is somewhat more florid, but no signs of ischemic parenchymal or interstitial injury are detected. **C**, subendothelial infiltration of lymphocytes in the portal veins and, **D**, infiltration and damage of small bile ducts (*arrow*) are characteristic and diagnostic features. **E**, severe acute cellular rejection is diagnosed when there is evidence of acute rejection-related ischemia, such as interstitial hemorrhage, necrosis, and cell dropout (*pt* = portal tract; *cv* = central vein), or **F**, inflammatory or necrotizing arteritis. (From Demetris AJ, Qian S, Sun H, et al: *Am J Surg Pathol* [in press]. Used by permission.)

seen (see Fig 54). Lymphocytic arterial inflammation can occur but is present in less than 5% of cases of acute cellular rejection.<sup>447</sup> Inflammatory arteritis may be a component of rejection, but the vessels most commonly affected are the second- and third-order branches of the hepatic artery in the hilum, which are not accessible to needle biopsy evaluation.<sup>144, 145, 424, 427, 428</sup> The rather low incidence of arteritis detected in needle biopsy samples may therefore be due to a sampling problem.<sup>144, 145, 424, 427, 428</sup>

Surprisingly little inflammatory cell infiltration into the hepatic lobule is seen during rejection. In fact, if significant lymphocytic hepatocellular injury is detected in biopsy samples, a de novo or recurrent viral hepatitis is more likely to be the cause of graft dysfunction. The relative restriction of the inflammation to the portal tracts during rejection may be the result of the functional anatomy of the organ, the localization and concentration of MHC antigens, and possibly the location of portal dendritic or capillary endothelial cells.<sup>409, 414</sup>

Fine needle aspiration biopsy (FNAB) sampling of the liver has been advocated as an adjuvant to the needle core for routine immunologic monitoring.<sup>448, 449</sup> Although this technique appears to be useful, it is diagnostically limited because no information is obtained on the architectural integrity of the organ, a problem that is of lesser significance in kidney grafts where FNAB is more routinely used. In the liver, there are many more causes of graft dysfunction, complications, and morphologic manifestation of systemic derangements, which require attention to architectural detail.

The distributions of the MHC antigen in human livers is altered after transplantation presumably because of local secretion of lymphokines.<sup>450, 451</sup> Steinhoff and associates<sup>411</sup> and Gouw and colleagues<sup>238</sup> detected a weak expression of class I antigens on hepatocytes early after transplantation in the absence of graft pathology. So and co-workers attributed this early presence to hepatocyte necrosis from harvesting injury.<sup>412</sup> Weak class II antigen expression was detected locally on bile ducts in the absence of cellular rejection. During rejection, class I antigens are upregulated on hepatocytes and bile ducts, and DR, DP, and at times DQ can be detected on biliary epithelia and endothelial cells.<sup>238, 409-413</sup> Steinhoff and associates were also able to detect weak DR expression on hepatocytes during rejection and viral infection.<sup>411</sup> Although several investigators have detected an association of an altered display of MHC antigen with certain graft syndromes, the patterns per se were not generally specific for any particular cause of dysfunction. Alterations have been detected during large duct obstruction and hepatic or systemic (or both) viral and bacterial infections, in addition to rejection.<sup>238, 409-413</sup>

Pathologic grading of acute cellular rejection is a controversial area. Several classifications systems have been proposed, but none is able to predict the likely response to therapy or eventual outcome

based on the initial histologic appearance unless irreversible damage, such as duct loss or obliterative arteriopathy, is already manifest.<sup>118, 431, 452</sup> Currently, the system in use in Pittsburgh adapts concepts applied to kidney and heart grafts (Table 9).<sup>144, 145</sup> As in the extrahepatic organs, the separation from mild to moderate acute cellular rejection is somewhat arbitrary and is based on the exuberance of the inflammation, which may have little prognostic significance. Severe rejection, on the other hand, is diagnosed when there is histologic evidence of rejection-related vascular compromise (ischemic damage), interstitial hemorrhage, and/or arteritis. The problem with applying such a system in the liver is that arteritis is rarely observed in biopsy samples, and apparent ischemic parenchymal changes may be nonspecific or unrelated to rejection. It may be that a sequential analysis of serial biopsies demonstrating continual deterioration is more predictive.<sup>431, 452</sup>

Functional analysis of lymphocyte cultures derived from rejecting human liver tissues demonstrates both proliferative and cytotoxic reactivity directed at donor MHC antigens.<sup>339, 453</sup> The concept of in vitro expansion of graft-infiltrating lymphocytes, which was discussed earlier (see Figs 50–54) in connection with drug development, is based on the fact that the T cells activated in vivo express growth-promoting IL-2 receptors, and in vitro, the addition of IL-2 to the culture medium selectively expands the activated cells. Both the proliferative and cytotoxic activities observed in lymphocyte cultures can be blocked by specific monoclonal antibody directed at class II or class I antigens.<sup>339, 453</sup> Functional analysis of the lympho-

**TABLE 9.**

Histopathologic Grading System of Acute Cellular Rejection

| Grade              | Histologic Findings   |
|--------------------|---|
| 1. Consistent with | Mononuclear portal interstitial infiltrate with "blastic" lymphocytes but little evidence of tissue damage*   |
| 2. Mild            | Mild predominantly mononuclear portal tract infiltrate with evidence of bile duct damage with or without subendothelial inflammation  |
| 3. Moderate        | Portal expansion secondary to predominantly mononuclear inflammation with duct damage and spillover into the lobule with or without periportal hepatocyte necrosis; no evidence of arteritis, central or bridging necrosis (rejection-related ischemia) |
| 4. Severe          | Usually marked but variable portal inflammation with evidence of interstitial hemorrhage and/or ischemic hepatocyte necrosis or inflammatory arteritis, in addition to findings in no. 2 or 3   |

\*Diagnosis used most often in the first 3 posttransplant weeks when there is clinical and biochemical evidence of graft dysfunction but histologic findings are not diagnostic (see text).

cytes from a needle core sample adds an informational dimension that, at present, cannot be gained from a strictly morphologic analysis.

In general, lymphocyte outgrowth from the biopsy specimen correlates well with the histologic diagnosis of moderate or severe acute cellular rejection.<sup>339, 453, 454</sup> However, alloactivated cells can be generated from biopsy specimens where the etiology of graft dysfunction is due to viral hepatitis.<sup>453</sup> The significance of this latter observation has yet to be determined. Similar studies have been performed in rat liver allografts.<sup>455</sup>

### *Chronic Rejection*

Recipients who develop chronic rejection usually experience a relatively asymptomatic rise in the canalicular enzymes (alkaline phosphatase and  $\gamma$ -glutamyl transpeptidase) and eventually become jaundiced.\* Although the term chronic implies a temporally prolonged course, this syndrome can evolve within weeks after transplantation or be the end result of acute rejection unresponsiveness to conventional therapy. Unfortunately, some of the patients will recapitulate the same course after retransplantation of a new graft.<sup>144, 145, 456, 457</sup> Synthetic function usually remains intact until late in the course, although rapid deterioration can occur in patients who develop superimposed vascular thrombosis or biliary tract stricturing and subsequent cholangitis.<sup>145</sup> Clinical suspicions of chronic rejection can be confirmed or ruled out after needle biopsy evaluation.<sup>144, 145, 424, 456, 457</sup>

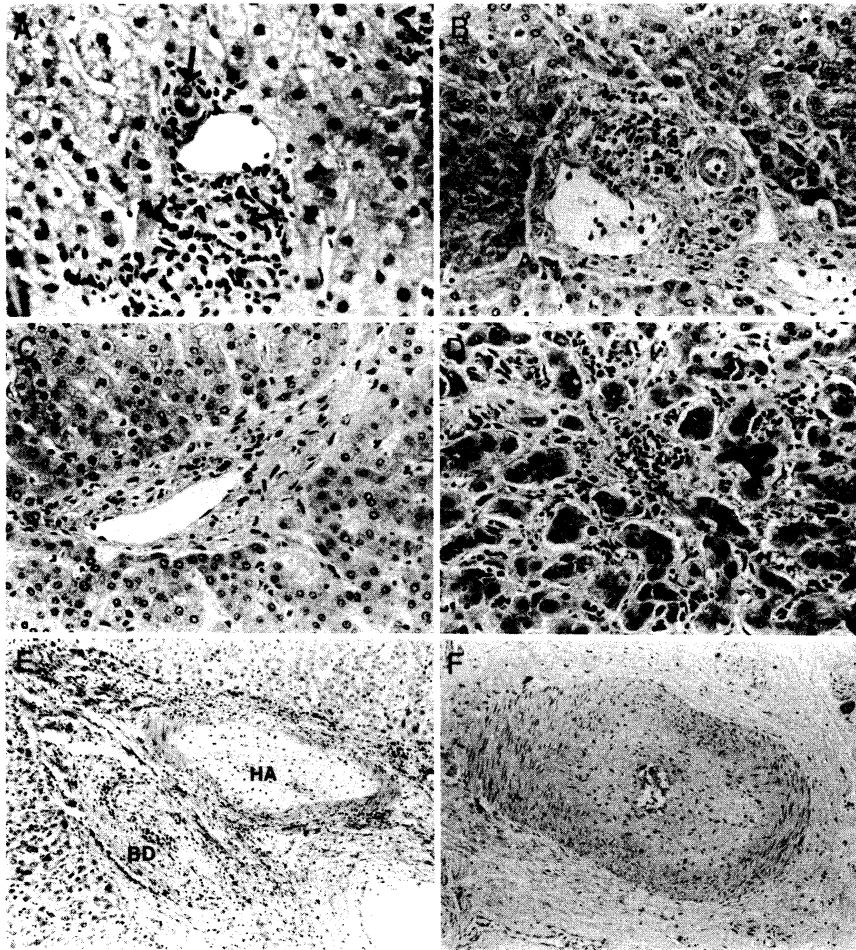
Occlusive arteriopathy and bile duct loss (vanishing bile duct syndrome) are the principal structural consequences of this form of immunologic graft injury.<sup>†</sup> Although these cardinal manifestations may occasionally appear to occur in isolation, we have shown a close relationship between the degree of arterial luminal narrowing and the severity of bile duct loss.<sup>458</sup> This dependency is not surprising considering the arterial system is the only source of blood for the bile ducts.<sup>146</sup> This led us to suggest that two mechanisms are responsible for the bile duct loss seen with chronic rejection: direct immunologic damage and ischemia.<sup>458</sup> The Cambridge group has also shown that disparity at the class I MHC locus (see the discussion of the effect of Histocompatibility) and CMV infection were interdependent predisposing factors for chronic rejection.<sup>459, 460</sup> In addition, patients with a positive pretransplant or posttransplant lymphocytotoxic crossmatch more commonly developed bile duct loss.<sup>459, 461</sup>

The histopathologic features of chronic rejection are somewhat

\*References 67, 144, 145, 423, 424, 433, 434, 456, 457.

†References 144, 145, 423, 424, 433, 434, 456, 457.





**FIG 55.**

Histopathologic features of "chronic" liver allograft rejection. **A**, in the earliest phases of chronic rejection, the portal infiltrate is often unimpressive, but the duct damage is severe (arrows). **B**, eventually the bile ducts are totally destroyed, a finding recognized by the presence of a portal artery without an accompanying bile duct. **C**, finally, the artery may be destroyed as well, and the portal triad becomes devoid of inflammatory cells. **D**, lobular changes include central hepatocanicular cholestasis, perivenular fibrosis, cell dropout, and mononuclear infiltration with occasional clusters of sinusoidal foam cells (cv = central vein). **E**, large septal arteries (HA = hepatic artery) become occluded, usually by subintimal foam cells, which causes ischemic injury and epithelial sloughing to accompanying septal ducts (BD = bile duct). **F**, fibrointimal hyperplasia similar to the arteriopathy in kidney and heart grafts can also be seen. (From Demetris AJ, Qian S, Sun H, et al: *Am J Surg Pathol* [in press]. Used by permission.)

subtle and easily overlooked if one is not attuned to recognizing its features (Fig 55). In contrast to acute cellular rejection, the portal infiltrate is often quite sparse and is comprised of lymphocytes, plasma cells, and macrophages. Acute inflammatory cells are uncommon. Despite the relative paucity of portal inflammation, epithelial cell pyknosis, disruption of the basement membrane, and complete destruction of small bile ducts ensue.<sup>†</sup> This response suggests that the effector mechanisms are extremely potent, noncellular, or ischemic in nature.

Lobular alterations include Kupffer's cell hypertrophy, mild spotty acidophilic necrosis like that seen in a low-grade lobular hepatitis, and eventual central hepatocanicular cholestasis. Small clusters of intralobular foam cells and perivenular hepatocellular atrophy, ballooning or dropout, and hemorrhage with sclerosis, presumably a result of chronic ischemia, are end-stage features. Although bridging fibrosis is occasionally seen, a cirrhosis with regenerative nodularity is uncommon.

The obliterative arteriopathy that develops does so most commonly in the branches of the hepatic artery in the hilum, vessels not routinely sampled in needle biopsies.<sup>145, 423, 424, 452-458</sup> Most affected arteries are narrowed because of deposition of subendothelial foam cells in the intima, the majority of which appear to be derived from recipient macrophages. However, the presence of T lymphocytes and interdigitating reticulum cells can also be seen in the intima media and periadventitia,<sup>462</sup> suggesting that cellular immunity is involved in the development of these lesions. Concepts from the response to injury hypothesis used to describe the development of atherosclerosis in the general population<sup>463</sup> appear particularly relevant to the obliterative arteriopathy that occurs in the transplant population.

#### ***Graft-vs.-Host Disease***

Control of the rejection may not be the only requirement for recipient survival. There has been increasing awareness that hepatic grafts can mount a significant attack on their recipient. The most likely explanation is the persistence of donor lymphoid tissue in the liver grafts.<sup>423, 464, 465</sup> The presence and continued viability of such donor lymphoid implies the possibility of GVH disease, a potential that has been documented by the demonstration of new circulating donor-specific Gm types in the recipient<sup>237, 464, 465</sup> and by the hemolysis caused by antihost RBC isoagglutinins, which are produced by the lymphoid tissues in ABO-compatible but not identical livers (e.g., O donor to A recipient).<sup>466, 467</sup> In addition, GVH disease has been reported in a recipient whose own tissue contained donor monocytes.<sup>468</sup> Intensification of immunosuppression relieved a skin rash, fever, and other symptoms of GVH disease. Unexplained wasting of a

febrile postoperative liver recipient who has a skin rash should cause GVH disease to be expected. A skin biopsy specimen should be obtained. Although continued viability of donor lymphoid cells has been documented in the cases cited previously, replacement of the donor lymphocytes in grafted hepatic hilar lymph nodes has also been shown.<sup>469</sup>



# INFECTIOUS PROBLEMS IN LIVER TRANSPLANTATION

## BACTERIAL AND FUNGAL INFECTIONS

Although liver grafts may possess some immunologic advantage, as discussed earlier, the practical reality is that heavy initial immunosuppression and later maintenance therapy are required in the same way as with other organs. The balance between immunosuppression and infectious disease control is more delicate than with cardiac and renal transplantation because the hepatic graft is exposed to the intestinal tract through the biliary tract or by hematogenous contamination from the splanchnic venous bed. The devastating role of consequent graft infection by organisms indigenous to the gastrointestinal (GI) tract was delineated in the early clinical trials<sup>18, 470-472</sup> as well as those in the cyclosporine era.<sup>358, 436, 473-475</sup> Experiments in dogs performed 25 years ago provided an example of what now is called *bacterial translocation* in that the liver graft itself became a porous entry site for bacteria indigenous to the GI tract.<sup>476</sup> A liver damaged by rejection becomes unusually vulnerable to invasion by such microorganisms. Effective immunosuppression has long been recognized to be the only way to maintain intact tissue barriers and to avoid this kind of infection.<sup>18</sup>

There has been recent interest in controlling the bacterial and fungal population of the GI tract with preoperative nonabsorbable oral antibiotics.<sup>252, 477</sup> These antibiotics selectively suppress pathogenic gram-negative organisms and fungi but allow survival of anaerobes. This has been called *selective intestinal decontamination*. A typical antibiotic regimen consists of polymyxin E, gentamycin, and nystatin. The morbidity from infection after liver transplantation has been reduced with this approach, but the mortality has not.<sup>252</sup> In addition to its unproved value, a practical limitation of selective decontamination is the inability to find a cadaveric liver at the optimal time ordained by the antibiotic preparation.

Much about the subtle relationships between host defenses and invasive bacteria remains to be learned in the liver transplant model. The host macrophage system, of which the liver is an important component,<sup>218</sup> is profoundly altered by transplantation. The possible role of altered graft Kupffer's cells in contributing to endotoxemia was discussed in an earlier section.

Liver recipients also suffer frequently from virus infections. The recurrence of hepatitis viruses in grafts will be discussed in the next section. Other virus infections occur at some postoperative time in the majority of liver recipients.<sup>478</sup>

#### CLINICOPATHOLOGIC FEATURES OF ALLOGRAFT VIRAL HEPATITIS

Clinical symptoms, along with the use of core biopsy, are used to establish the diagnosis of allograft hepatitis. In general, the clinical features and histologic appearance of allograft viral hepatitis are identical to those observed in other immunosuppressed patients. It is helpful, however, to anticipate the relative time of onset of the different viral syndromes, since they tend to occur at characteristic times after liver replacement (Table 10).<sup>\*</sup> The following sections are separated into discussions of those viruses that are classically associated with hepatitis from those that are more opportunistic in nature.

##### *Opportunistic Viruses*

The most common viral pathogens in the opportunistic category that cause allograft hepatitis belong to the herpes family: CMV, HSV types 1 and 2, varicella-zoster (VZ) virus, and EBV. Another cause of allograft hepatitis not commonly seen in the general population is adenovirus (ADV). The following are presented in order of frequency.

##### *Cytomegaloviral Hepatitis*

The most common serious infections are with CMV, which can cause lesions in many organs.<sup>353, 354, 478-480</sup> Cytomegalovirus is the most common cause of postoperative graft hepatitis and is seen most frequently between 3 and 8 weeks after transplant.<sup>353, 354, 478-480</sup> Protection from serious CMV infection has been reported with hyperimmune globulin.<sup>481</sup> Recovery is the rule if im-

**TABLE 10.**

Peak Incidence of Graft Syndromes vs. the Time After Transplant

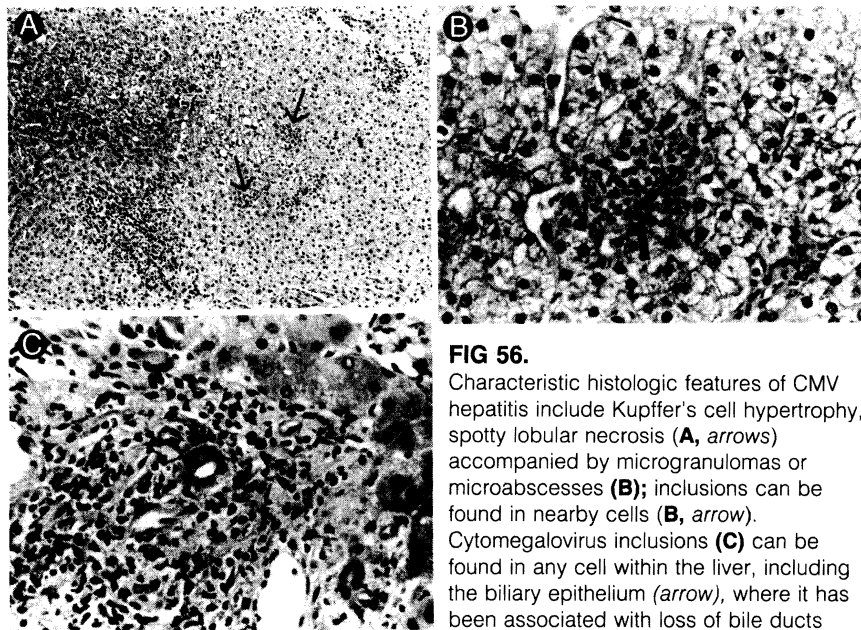
| Viral Syndromes        | Time After Transplant                                       |
|------------------------|---|
| Cytomegalovirus        | 3-8 wk, often after treatment of rejection                  |
| Herpes simplex         | Any time after transplant                                   |
| Epstein-Barr           | Most common in first 2 mo. but may occur anytime thereafter |
| Adenovirus             | 3-4 wk after transplant.                                    |
| Hepatitis B            | Onset usually after 4-6 wk, and graft remains infected      |
| Hepatitis A            | No experience to date                                       |
| non-A, non-B hepatitis | Usually after 4 wk  |

<sup>\*</sup>Table 1 appears in Part I; Tables 2-9 appear in Part II.

munosuppression is lightened and especially if therapy is given with ganciclovir (Gancyclovir).<sup>479, 482</sup> However, CMV strains resistant to ganciclovir have been reported recently.<sup>483</sup> The onset of CMV is often temporally related to episodes of rejection, where the patient has just received additional immunosuppressive therapy for an acute cellular rejection episode.<sup>353, 354, 478–480</sup>

Clinically, patients usually present with a low-grade fever and mildly elevated liver injury test results. Leukocytopenia, diarrhea, GI ulcers, and respiratory symptoms are not uncommon.<sup>353, 354, 478–480</sup> The diagnosis of liver involvement is confirmed by needle biopsy.<sup>144, 145, 353</sup>

Cytomegaloviral hepatitis is characterized by lobular alterations (Fig 56).<sup>\*144, 145, 353</sup> Any cell type of the liver may be infected, and those that are may demonstrate cytomegalic change, intranuclear eosinophilic inclusions surrounded by a halo, and/or small basophilic cytoplasmic inclusions. These foci are often infiltrated with clusters of inflammatory cells, consisting of neutrophils, macrophages (microabscesses and microgranulomata), or both. Other lobular alterations include mild Kupffer's cell hypertrophy. Significant lobular disarray, massive or submassive necrosis, or even severe liver damage from CMV alone is rare. Recognition of any of these changes should prompt a careful search for viral inclusions, the use of immu-



**FIG 56.**

Characteristic histologic features of CMV hepatitis include Kupffer's cell hypertrophy, spotty lobular necrosis (**A**, arrows) accompanied by microgranulomas or microabscesses (**B**); inclusions can be found in nearby cells (**B**, arrow). Cytomegalovirus inclusions (**C**) can be found in any cell within the liver, including the biliary epithelium (arrow), where it has been associated with loss of bile ducts (see text).

\*Figures 1–42 appear in Part I; Figures 43–55 appear in Part II.

nohistochemical stains for the detection of the CMV antigens, or both.

Tissues containing rapidly dividing cells, such as young granulation tissue, proliferating cholangioles, edges of infarcts, and abscesses or other defects are fertile soil for CMV growth.<sup>144, 145</sup> When such tissue is encountered, a more careful search of CMV is warranted.<sup>144, 145</sup>

Finally, CMV can be associated with a plasmacytoid or blastic infiltrate (or both) similar to that seen in EBV hepatitis (unpublished observations). Cytomegalovirus inclusions are not usually detected in such cases. Differentiation from rejection and lymphoproliferative disease associated with EBV may be difficult and is based on careful microscopic examination and immunohistochemical stains to detect viral antigens. The clinical profile and various hematologic parameters are also helpful.

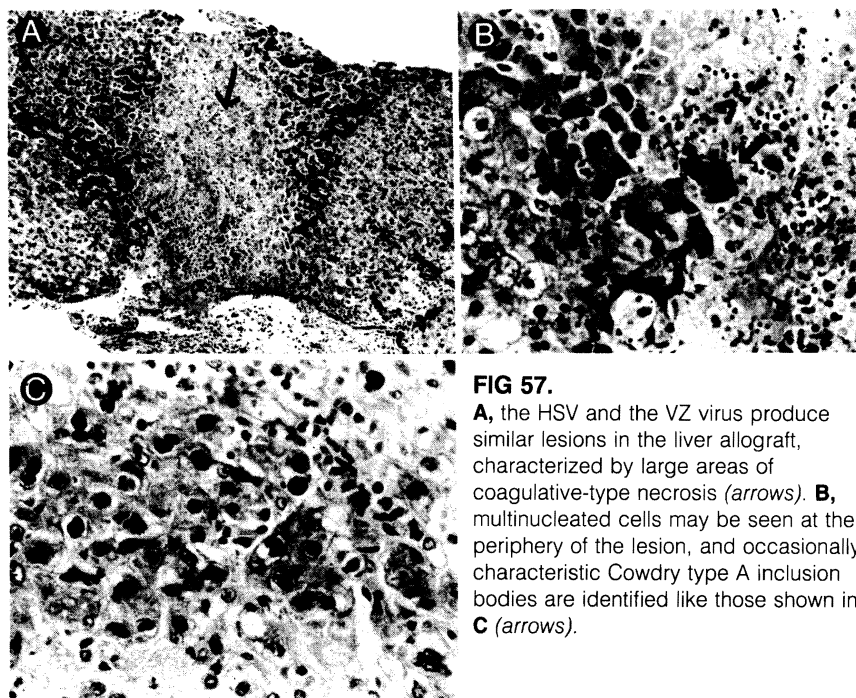
Recently, CMV has been implicated in the pathogenesis of the vanishing bile duct syndrome (VBDS).<sup>460</sup> Compatibility between the donor and recipient at the DR MHC locus, along with mismatching at the class I locus and CMV infection have been identified as interdependant risk factors for the development of bile duct loss.<sup>459, 460</sup> The Cambridge group has suggested that MHC-restricted antigen presentation of viral antigens or mismatched class I MHC antigens by DR-compatible bile duct cells is responsible for this observation.<sup>460</sup>

### *Herpes Simplex and Varicella-Zoster Hepatitis*

Both subtypes of HSV (1 and 2) and the VZ virus have been identified as causes of liver allograft hepatitis. Signs of graft infestation have been seen as early as 3 days after transplant and may occur any time thereafter.<sup>144, 354, 484</sup> The clinical presentation with the HSVs includes fever, fatigue, and body pain combined with serologic evidence of hepatic injury.<sup>144, 145, 354</sup> Cutaneous manifestations may or may not be present. With the VZ virus, allograft involvement may be detected several days prior to the eruption of cutaneous vesicles typical of this disorder. Untreated, any of these viruses may rapidly lead to massive hepatic necrosis. Therefore, early recognition on needle biopsy is particularly crucial since effective medical therapy (acyclovir) is available.

Microscopically, all three viruses produce similar graft pathology (Fig 57).<sup>144, 145, 484</sup> They are characterized by circumscribed areas of coagulative necrosis, showing no respect for the lobular architecture. Ghosts of hepatocytes intermixed with neutrophils and nuclear debris are seen in the center of the lesions. More viable hepatocytes are seen at the periphery, some of which may contain ground glass nuclei or characteristic inclusion bodies. Multinucleated cells are





**FIG 57.**

**A**, the HSV and the VZ virus produce similar lesions in the liver allograft, characterized by large areas of coagulative-type necrosis (*arrows*). **B**, multinucleated cells may be seen at the periphery of the lesion, and occasionally characteristic Cowdry type A inclusion bodies are identified like those shown in **C** (*arrows*).

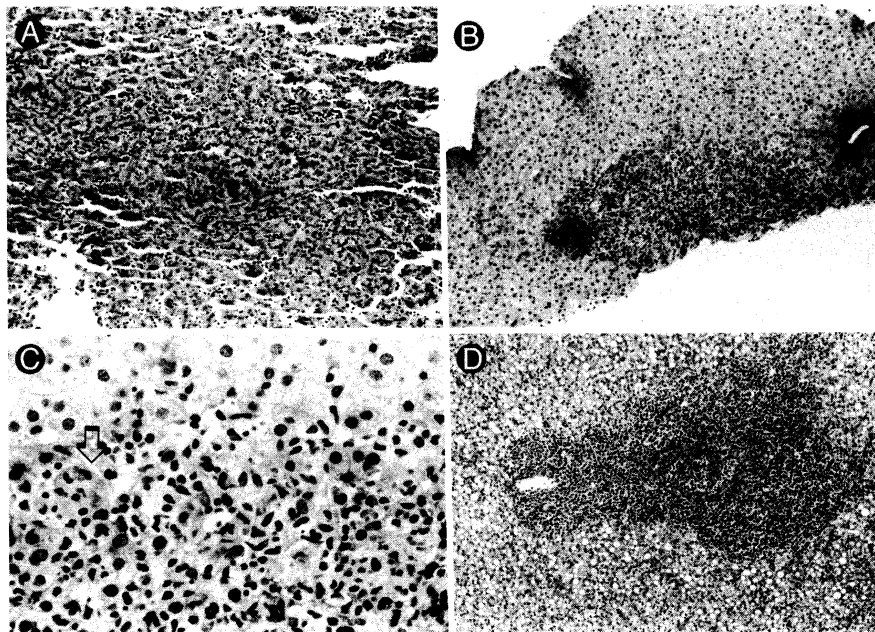
also occasionally present. Immunoperoxidase stains for various viral antigens confirm the diagnosis when the pathologist is unsure on the basis of the hematoxylin-eosin stains alone.

#### *Epstein-Barr Virus*

Consequences of primary infection or reactivation of the EBV after transplantation run the gamut from an infectious mononucleosis syndrome as seen in the general population<sup>485</sup> to severe life-threatening lymphoproliferative disease similar to patients with the X-linked lymphoproliferative disorder<sup>486</sup> or acquired immunodeficiency syndrome (AIDS).<sup>487</sup> Lymphoproliferative tumors (B-cell lymphomas) have been seen with all kinds of transplantations but most frequently in liver recipients<sup>355, 488–491</sup> and especially in infants and children, in whom the risk over the first 2 years after transplantation may be as high as 10%.<sup>355, 492</sup> The liver graft itself is frequently involved. The most effective treatment measure for any of the EBV syndromes is discontinuance or reduction of immunosuppression,<sup>488</sup> to which antiviral therapy with acyclovir should be added.<sup>493</sup> Regression of the symptoms, laboratory abnormalities, and lymphomas usually, although not invariably, follows reduction of immunosuppression whether or not acyclovir is given.<sup>488, 490, 491</sup> This effect

may be achieved even though the hepatic graft is not rejected. The regression of these lymphomas, some of which are monoclonal, when the recipient immunologic responsiveness is allowed to recover is thought to be an example of immunologic surveillance in humans.<sup>488</sup>

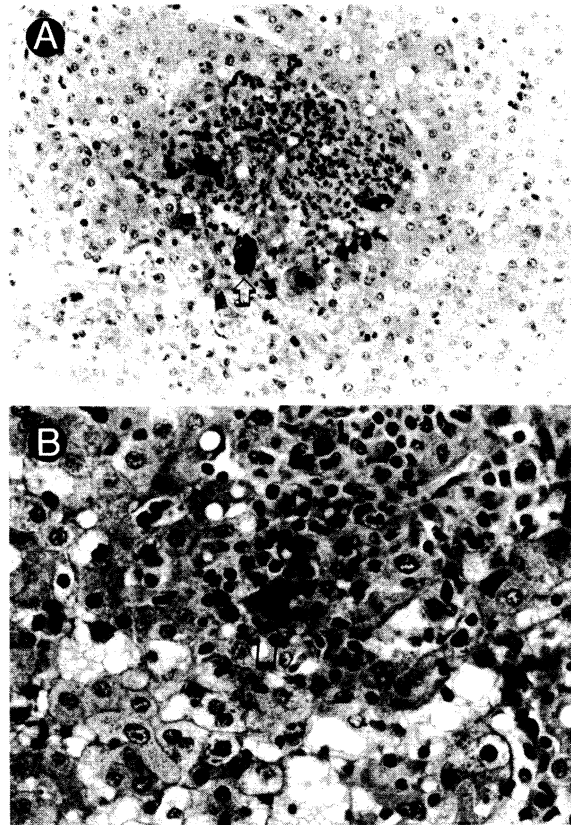
Clinical signs and symptoms of recipients with EBV syndromes at the more benign end of the spectrum are similar to those seen with infectious mononucleosis, although atypical presentation in the form of fever, rashes, and joint and jaw pain are not uncommon. Liver enzyme levels are usually only modestly elevated, but occasionally significant damage and even submassive or massive necrosis may be seen. Those recipients who develop tumors present clinically with constitutional symptoms similar to those just described in addition to those related to organ system involvement with tumor.<sup>488–491</sup> Atypical lymphocytosis in the peripheral blood smear is invariably present in all patients. The diagnosis of allograft involvement is confirmed by needle biopsy evaluation of the graft.



**FIG 58.**

The EBV causes a spectrum of pathologic lesions in the liver, ranging from mild lobular hepatitis with sinusoidal lymphocytosis (**A**) to granulomatoid collections (**B**) of immunoblastic lymphocytes, which can be associated with hepatocyte necrosis (**C**, arrow). Epstein-Barr virus–driven lymphoproliferative lesions in the liver (**D**) are characterized by a monomorphic infiltrate that overruns the normal architectural landmarks. (From Demetris AJ, Jaffe R, Starzl TE: *Pathol Annu* 1987; 22:347–386. Used by permission.)

Like the variety of clinical disorders, involvement of the liver by EBV-associated disorders also runs the histopathologic gamut from typical monohepatitis as seen in the general population to submassive or massive hepatic necrosis<sup>145</sup> or involvement by tumor, comprised of malignant lymphoid cells similar to those seen in immunoblastic lymphomas (Fig 58). Cases resembling lymphomatous involvement of the liver may be difficult to differentiate from acute cellular rejection<sup>145</sup> since subendothelial infiltration of the portal veins along with focal bile duct damage may be present. Usually these are not as severe or as widespread as those seen with rejection. The key to the diagnosis is the monomorphic and atypical appearance of infiltrative cells in the EBV-related disorders. Immunohistochemical



**FIG 59.**

**A**, the ADV causes typical granulomas in the liver. Immunoperoxidase stains can be helpful if one cannot identify the inclusion bodies (*arrows*). **B**, at the periphery of the granulomas, infected cells with intranuclear inclusions appear smudgy. (From Demetris AJ, Kaki-zoe S, Oguma S: Pathology of liver transplantation, in Williams JW [ed]: *Hepatic Transplantation*. Philadelphia, WB Saunders Co [in press]. Used by permission.)

staining to detect EBV viral antigens can be performed but requires frozen tissue. Immunophenotypic analysis of the infiltrative cells in EBV-related disorders usually demonstrates a great number of non-T cells, whereas in acute cellular rejection, the T cells predominate.

Biopsy of enlarged lymph nodes (most common) or other organs infiltrated by tumor is also used to establish the diagnosis of an EBV-related disorder. In the nodes, the changes vary from those seen with infectious mononucleosis<sup>494</sup> to a histology indistinguishable from immunoblastic lymphoma.<sup>489</sup> Immunohistochemical and light-chain immunoglobulin gene rearrangement analysis are used to establish the clonality of the tumors, if present.<sup>488-491</sup>

### *Adenoviral Hepatitis*

Allograft hepatitis due to the ADV has been restricted to primarily the pediatric population, although more recently an unequivocal case in an adult has been identified.<sup>356, 357</sup> Adenovirus usually occurs within a very narrow time frame, namely, 20 to 30 days after transplant, and the patients present with fever and elevated liver injury test results.<sup>357</sup> To date, almost all of the cases of ADV in the transplant population have been caused by viral subtype 5.<sup>357</sup> However, other viral subtypes (2, 11, and 16) have been associated with hepatitis in the general population and could be expected to infect allografts.<sup>495</sup> The diagnosis is made on needle biopsy sampling of the organ,<sup>357</sup> after which immunosuppression should be temporarily stopped.

Histologically, granulomatoid collections of histiocytic cells are randomly located throughout the parenchyma (Fig 59). Hepatocyte necrosis may be detected but usually is less severe than that seen with HSV. Characteristic "smudgy" intranuclear inclusions can be identified in hematoxylin-eosin-stained sections, but experience is required to be confident of the diagnosis without the use of special stains. In infected cells, the chromatin is crowded toward the nuclear membrane, which imparts a muffin-shaped appearance to the nucleus. Immunohistochemical stains are confirmatory.

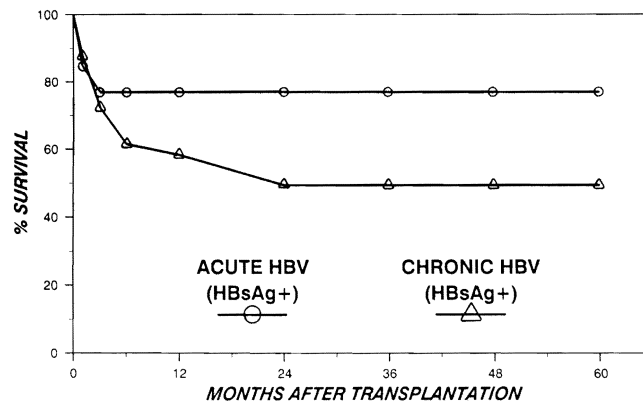
# HEPATITIS VIRUSES

## *HEPATITIS B VIRUS*

Viral hepatitis type B in the posttransplant period is restricted largely to those patients who carried the virus prior to transplantation, although a few patients have acquired an infection, presumably as a result of blood transfusion. Provision of a new liver usually, but not always, lowers the titer of the virus, as measured by the surface antigen,<sup>496, 497</sup> but return of the carrier state is almost universal.<sup>498–502</sup> In spite of this generalization, some chronic carriers have apparently cleared the virus after transplantation<sup>499–503</sup> with passive immunoprophylaxis. In our experience, those chronic carriers who have cleared the virus have been E antibody positive and E antigen negative, although this serologic profile is no guarantee that infection will not recur. Among those recipients who become reinfected, a small percentage will develop a carrier state and experience long-term survival with minimal liver dysfunction. Recapitulation of the original chronic aggressive hepatitis jeopardized the recovery of many of the recipients.<sup>496–499, 501</sup> Delta agent coinfection is an additional confounding factor and recurs along with the B virus.<sup>497, 500, 501</sup> Reinfection of the allograft after transplantation for acute fulminant hepatitis B is less certain, with several patients experiencing long-term survival with viral immunity.<sup>497, 498</sup> The survival with acute disease and fulminant hepatic failure has been acceptable, although less favorable with chronic disease (Fig 60).

In those who develop HBV disease after liver replacement, the onset of symptoms usually occurs 6 to 8 weeks after transplantation. The presentation varies from asymptomatic elevations of liver injury test results to nausea, vomiting, jaundice, and hepatic failure. The clinical syndrome, therefore, is not significantly different from viral hepatitis as seen in other immunosuppressed hosts. Serologic evaluation and needle biopsy of the graft confirm the diagnosis.

Pathologic identification of acute hepatitis B as a cause of dysfunction rests on the recognition of preferential lobular alterations in the absence of significant inflammatory cell damage to bile ducts, arteries, and venular endothelia.<sup>497</sup> However, the pathologic appearance of HBV in the allograft is as varied as the complete spectrum of acute and chronic viral hepatitis as seen in the general population (Fig 61).<sup>145</sup> Simply stated, viral hepatitis in the liver allograft looks like



**FIG 60.**

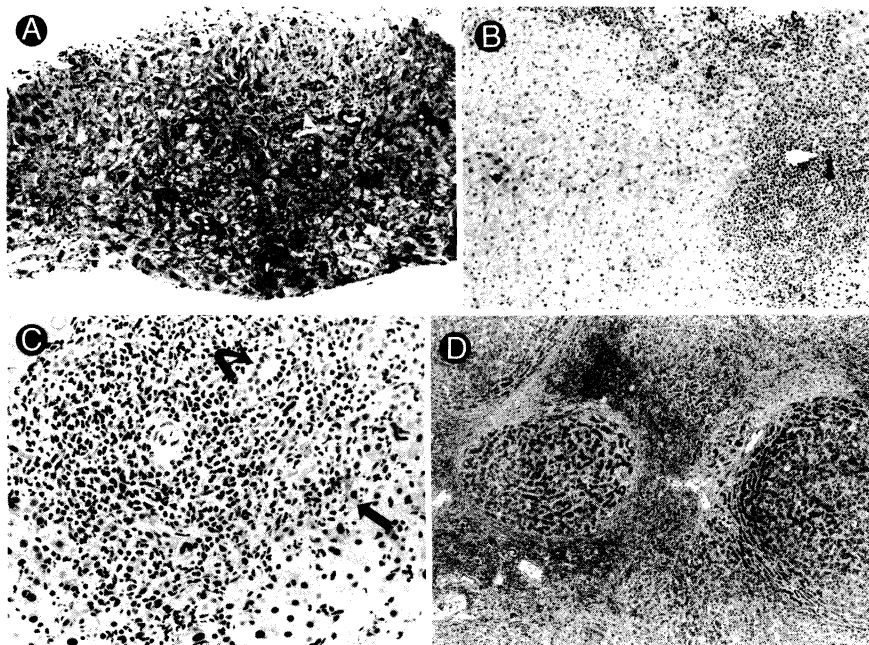
Patient survival (life table method) after liver transplantation with cyclosporine-prednisone for 65 adults with chronic B virus hepatitis compared with 13 adults with acute B virus hepatitis.

viral hepatitis in other livers except for a relative paucity of inflammation in some cases, even with severe clinical manifestations and pathologic changes.

The natural history of hepatitis B infection of the allograft liver is becoming clearer. In our series of 59 patients who received allografts because of HBV disease, pathologic follow-up was available in 39 of 46 recipients who survived for more than 60 days. Thirty-four of these 39 patients had histologic evidence of recurrent hepatitis B infection, disease, or both.

A very typical sequence of pathologic changes was observed in these specimens. The first evidence of recurrent hepatitis B infection was the detection of hepatitis B core antigen in the cytoplasm of hepatocytes several weeks after transplantation. Little pathologic change was detected at this time. Several weeks thereafter, mild lobular disarray, hepatocyte swelling, and mild spotty acidophilic necrosis with regenerative change coincided clinically with the onset of elevated liver injury test results and signaled the development of disease activity. Most of the specimens at this time had the appearance of a mild acute hepatitis as seen in the general population except for a relative paucity of lobular portal inflammation.

Follow-up of these patients over several weeks to greater than 5 years revealed several clinicopathologic "syndromes." Six of the patients experienced a syndrome of unresolved lobular hepatitis, and five settled into a clinicopathologic profile resembling chronic carriers with little disease activity. Eighteen others developed chronic active hepatitis, and four of these became cirrhotic, 1.5 to 5 years after



**FIG 61.**

Hepatitis B virus infection of the allograft causes pathologic lesions similar to those seen in the general population and in other immunosuppressed hosts. In **A** there is an acute hepatitis with lobular disarray, hepatocyte ballooning, and necrosis. **B**, in chronic active B viral hepatitis in the allograft, a portal infiltrate with active piecemeal necrosis (*arrow* indicates intact bile duct) (**C**, *straight arrow*) and preservation of the bile ducts (*curved arrow*) are the identifying features. **D**, the eventual outcome of many cases with chronic active hepatitis after transplantation is graft failure or cirrhosis, which may occur with surprising rapidity (see text).

transplant. A fifth patient rapidly became cirrhotic 147 days after liver replacement without any evidence of intervening chronic active hepatitis after transplantation. Follow-up of the few patients who have apparently cleared the virus with no serologic or histologic evidence of recurrent B viral infection of the liver revealed nonspecific changes in three, non-B chronic active hepatitis in one and acute cellular rejection, which responded to bolstered immunosuppressive therapy, in the remaining patient.

It is not always easy for the pathologist to distinguish between rejection and hepatitis as a cause of malfunction. The most useful feature overall used to differentiate these two causes of malfunction is the focus of lymphocytic damage. The bulk of the injury associated with acute HBV is directed at hepatocytes and is recognized as lobular alterations. Acute rejection, on the other hand, is directed at structures within the portal tracts. In chronic hepatitis, portal in-

flammation is present, and lobular alterations may be minimal. In these cases, one has to determine if piecemeal necrosis or bile duct destruction is the more prominent feature. It must be stressed that an overall assessment of the entire biopsy specimen with careful examination of each portal tract must be performed. Individual cases may be quite difficult since both bile duct damage and significant piecemeal necrosis may be present. It has been our policy that if a significant amount of duct damage is detected, regardless of the presence of piecemeal necrosis, a diagnosis of rejection made. A therapeutic or diagnostic clinical trial of immunosuppressive therapy is then initiated. This approach seems prudent, considering the fact that reductions of immunosuppression during hepatitis B infection may result in fulminant liver failure.

#### *NON-A, NON-B HEPATITIS*

Although precise identification of at least one of two viruses responsible for non-A, non-B hepatitis has just recently been achieved (hepatitis C),<sup>504</sup> it is undoubtedly a cause of allograft hepatitis.<sup>144, 145, 505</sup> Episodes in patients with cryptogenic cirrhosis, in those with unrelated disorders, and in patients who were thought to have the disease prior to transplantation have been identified. It may therefore be recurrent or develop *de novo*. The onset of symptoms and laboratory abnormalities usually appear after 6 weeks. The clinical presentation is as variable as that seen in the general population: mild asymptomatic elevation of liver injury test results to massive hepatic necrosis. Bone marrow aplasia, which also can complicate milder attacks of non-A, non-B hepatitis not requiring liver transplantation,<sup>506, 507</sup> has been observed in children a few days or weeks after liver replacement.<sup>508, 509</sup> Four of the nine patients with marrow aplasia survived, usually with slow recovery of the hematopoietic system.<sup>508, 509</sup> At present, the diagnosis is based largely on biochemical evidence of liver injury combined with the histopathologic profile, although supporting serologic data may soon become available.

The histopathologic appearance of presumed non-A-, non-B hepatitis may be as varied as that described for hepatitis B earlier. Needle biopsy specimens from patients thought to be infected during the acute stages show mild Kupffer's cell hypertrophy, spotty acidophilic necrosis of hepatocytes, and a relative paucity of inflammation. However, lobular disarray, mixed inflammatory cell infiltration, hepatocyte ballooning, and necrosis, which may be bridging, have also been seen. The disease may also recur in a more fulminant fashion, as was experienced with two patients in Pittsburgh, where the clinical profile and histologic appearance of the failed graft was



remarkably similar to the native organ. Later, features of chronic persistent or active viral hepatitis are not uncommon (Fig 62).

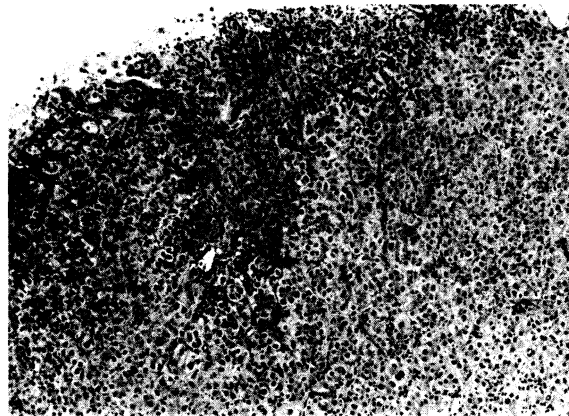
Pathologically, in acute disease the diagnosis is based largely on the lobular insult and is usually not difficult to differentiate from rejection. In chronic disease where the histologic appearance is that of chronic persistence or active hepatitis, it may be hard to differentiate from an indolent rejection reaction. It has been our policy that if there is evidence of significant duct damage, rejection is considered present.<sup>145</sup>

#### *HEPATITIS A VIRUS*

Although fulminant hepatitis A virus has been an indication for liver replacement, it has not as yet been identified as the cause of allograft dysfunction. Based on these observations, we expect that it may appear quite similar clinically and histologically to that seen in nongrafted livers.

#### **THE PATHOLOGIST'S VIEW OF BILIARY TRACT COMPLICATIONS**

Anastomotic breakdown, necrosis, strictures, ascending infection, and obstruction can affect the allograft biliary tree.<sup>84-90, 93</sup> Although these complications are not uncommon in isolation, they often re-



**FIG 62.**

The histologic appearance of presumed non-A, non-B viral hepatitis in the allograft is similar to the type B virus. In this case a chronic active hepatitic lesion is seen. (From Deme-tris AJ, Kakizol S, Oguma S: Pathology of liver transplantation, in William JW [ed]: *Hepatic Transplantation*. Philadelphia, WB Saunders Co [in press]. Used by permission.)

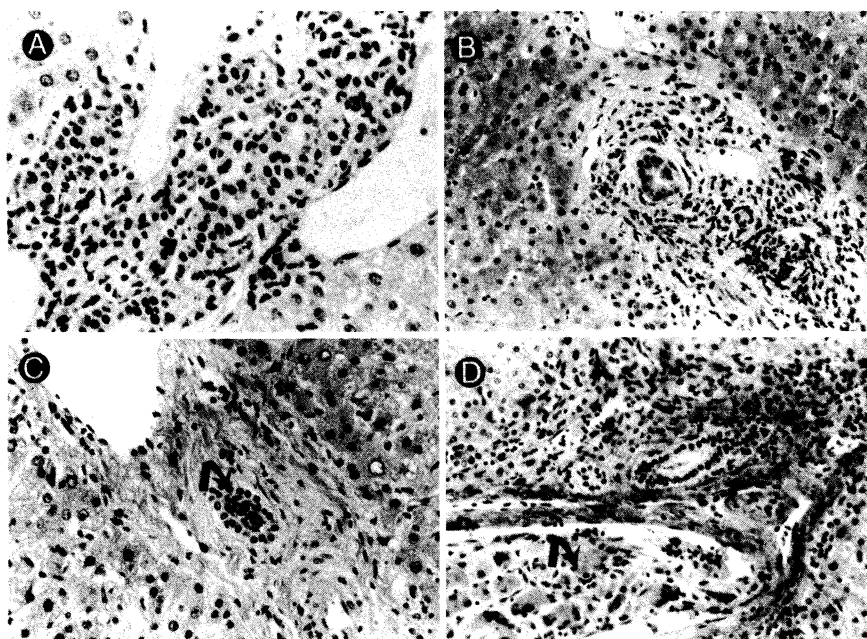
flect arterial pathology since the biliary tree is dependent solely on the hepatic artery for its blood supply.<sup>146</sup> Most often the diagnosis of biliary complications is made on the basis of clinical symptoms and the results of radiologic procedures such as ultrasonography and cholangiography (see previously).<sup>84-90</sup> In addition, during the early postoperative period, most patients have a percutaneous T tube in place that permits ready access to the biliary tree for radiologic procedures and assessment of bile flow.

Needle biopsies are less useful than radiologic evaluations for the diagnosis of large biliary tract disorders because of the relative nonspecificity and insensitivity of early histologic findings.<sup>144, 145</sup> However, when access to the biliary tree is restricted, (late posttransplant period), biopsies may be more valuable as a screening tool. Biliary tract complications that have been recognized histologically include duct stricturing, obstruction, acute cholangitis, and biliary-vascular fistulas.<sup>144, 145</sup> The histologic features of these complications are identical to those seen in the nonallograft liver (Fig 63), which include a predominantly neutrophilic portal infiltrate, periductal edema, intraepithelial and intraductal neutrophils, mild ductular and cholangiolar proliferation, centrilobular hepatocanicular cholestasis, and small clusters of neutrophils scattered throughout the lobules. Although acute cellular rejection is included in the pathologic differential, biliary tract disorders most commonly are associated with a neutrophilic and eosinophilic portal infiltrate, whereas rejection shows a predominance of mononuclear cells in the portal tracts.

Recognition of biliary-vascular fistulas may be first noticed by the pathologist on needle biopsies and requires alertness to the abnormal presence of RBCs in bile duct lumens or, conversely, bile concretions in blood vessels (see Fig 63). Radiologic localization of the abnormal communication, followed by corrective surgery or retransplantation, is the usual course of events.

## SEPSIS

Infection of the blood, especially with gram-negative organisms, can cause allograft dysfunction, which is usually manifested as jaundice. Histologic alterations are also observed in the graft as a result of sepsis (endotoxemia) and are identical to those seen in nonallograft livers.<sup>510</sup> These changes include cholangiolar proliferation with bile plugging, acute cholangiolitis usually without cholangitis, and hepatocanicular cholestasis. Kupffer's cells are often hypertrophied, and small clusters of neutrophils can be observed in the lobules.<sup>144, 145</sup>



**FIG 63.**

The histologic manifestation of biliary tract complications in the allograft are similar to those in nonallografted livers. The most important of these features is the neutrophilic predominance of the portal infiltrate in the absence of reactive biliary epithelial cell changes, as shown in this case of acute cholangitis (**A**). When the biliary tree is obstructed, periductal edema accompanies the acute portal inflammation, and cholestasis is present in the lobules (**B**). Fistulas between the biliary tree and the vasculature are recognized by the presence of RBCs in bile ducts (**C**, arrow) or bile concretions in blood vessels (**D**, arrow). (From Demetris AJ, Kakizol S, Oguma S: Pathology of liver transplantation, in William JW [ed]: *Hepatic Transplantation*. Philadelphia, WB Saunders Co [in press]. Used by permission.)

### DIFFERENTIAL DIAGNOSIS OF DRUG AND TOXIC INJURY

Drug and toxic injury to the allograft liver are difficult to identify with certainty. The patients receive many potential hepatotoxic drugs and are subjected to other therapeutic maneuvers that may damage the liver. Therefore, if one strictly adheres to criteria for organ specific toxicity, it is extremely difficult to incriminate any agent. Regardless of these difficulties, erythromycin, prolonged peripheral alimentation, high-dose steroids, and azathoprine have been strongly suspected as causes of allograft malfunction.<sup>144, 145</sup> One might expect the allograft liver to behave similar to nongrafted livers in regard to drug toxicities, unless an MHC-restricted immunologic reaction is involved.

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## INFLUENCE OF HISTOCOMPATIBILITY

Histocompatibility leukocyte antigen (HLA) or MHC compatibility has been shown to either improve patient survival or reduce the onset or incidence acute rejection in kidney<sup>511</sup> and heart allografts.<sup>512</sup> Data collected by Markus and associates concerning the role of HLA matching in liver transplantation were less clear cut.<sup>513</sup> No patient survival advantage was observed for HLA compatibility. By contrast, a statistically significant penalty in terms of survival was detected when either the A, B, or DR locus was matched. Although rejection as a cause of graft failure was more common when DR mismatching was present, other causes of patient death or graft failure were even more common when either class I or II loci were matched. Primary nonfunctioning of the new liver was particularly common in DR-matched grafts. However, the diagnosis "primary nonfunction" is somewhat of a wastebasket category, which often includes preservation injury, antibody-mediated rejection, vascular thrombosis, surgical misadventures, and cardiovascular instability in the donor or recipient. Markus and associates suggested that MHC compatibility may provide the ideal setup for recurrent disease since some of the immunopathologic mechanisms important in the native diseases are thought to be MHC restricted.<sup>513</sup> Alternatively, they suggested that the alloresponse itself may be MHC restricted. Donaldson and colleagues proposed a similar hypothesis.<sup>459</sup> They found that DR-matched but A and/or B locus-mismatched grafts were more prone to develop the vanishing bile duct syndrome (chronic rejection). They suggested that induction of DR antigens on bile duct cells enabled these cells to act as antigen-presenting cells, presenting the mismatched class I antigens in an MHC-restricted fashion to recipient effector cells.

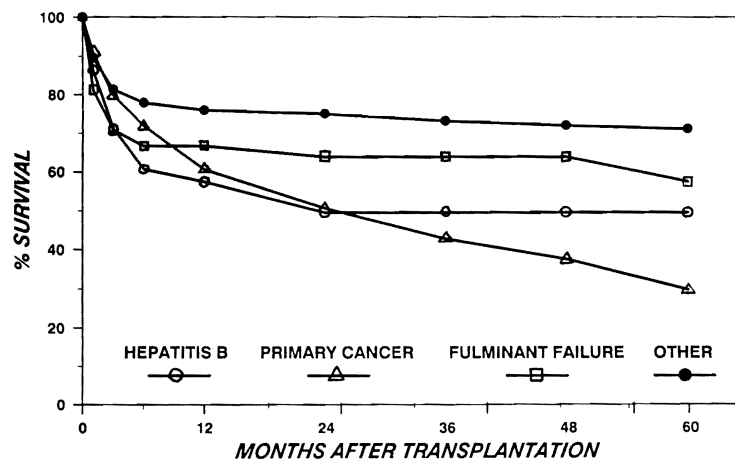
There are many possible explanations for the somewhat peculiar observations made with respect to HLA matching and liver allograft outcome. Like other allografts, livers seem to experience a lower incidence of rejection when the DR locus is matched. Paradoxically, there does not appear to be a patient or graft survival advantage for

DR or class I matching. This may be due to graft loss or patient death from causes other than rejection (e.g., technical mishaps and infection). A higher incidence of recurrent native disease in HLA-matched patients may be a possibility, since cellular "immune" mechanisms are thought to play a prominent role in native hepatic disease. This contrasts to most cardiac and renal diseases for which transplantation is performed, where cellular immunity is not strongly implicated. This argument is appealing because the immune damage purportedly mediated by T lymphocytes in liver diseases such as hepatitis B is thought to be MHC restricted. However, the pathogenic mechanisms responsible for many native liver diseases have yet to be elucidated. Furthermore, recurrent disease must be proved after liver grafting, which is not an easy task. Rather than to continue speculation, reanalysis of the data after collection of a much larger patient population seems wise.

## CANDIDACY, ORIGINAL DISEASE, AND OUTCOME

In spite of the diversity of etiologies, manifestations, and variability of technical problems with different diseases, the survival curves have not been greatly influenced by the original diagnosis with the exceptions of fulminant hepatic failure, chronic active hepatitis due to B virus, and liver malignancies (Fig 64).<sup>498, 499, 514-517</sup> These observations, which have been extensively documented, are analogous to those in renal transplantation where the original kidney disease has been said to have little influence on the outcome.

However, the foregoing summary is oversimplified, which could degrade the value of information summarized in the following pages that covers not only the influence of disease on outcome but also



**FIG 64.**

Comparison of patient survival rates (life table method) after liver transplantation in adults receiving cyclosporine-prednisone for HBsAG-positive postnecrotic cirrhosis (66 cases), primary hepatobiliary cancer (89 cases), fulminant hepatic failure (48 cases), and other nonmalignant indications for liver transplantation (827 cases).

many other factors, including the severity of the disease at the time of the liver replacement, issues of organ supply, and the role of socioeconomic factors. Thus, the serious student of hepatology, liver surgery, and liver transplantation is urged to read this section and not skip to the next one.

The medical issues of transplant candidacy are relatively clear. If a patient has end-stage nonmalignant liver disease that does not recur in the hepatic graft, there is little debate about the logic in principle of transplantation (Table 11). Transplantation is more debatable if recurrence of a nonneoplastic disease is a predictable problem. The most controversial indication for liver transplantation is for the treatment of hepatic malignancies. However, none of these broad applications can be arbitrarily excluded from future trials because there is such heterogeneity in each of these three categories.

In adults, the diseases most commonly represented have been postnecrotic cirrhosis, primary biliary cirrhosis, alcoholic cirrhosis, sclerosing cholangitis, inborn errors of metabolism, and a heteroge-

**TABLE 11.**

Indications for Liver Transplantation in 438 Pediatric and 1,031 Adult Patients

|                                  | Pediatric | Adult | Total | %     |
|----------------------------------|-----------|-------|-------|-------|
| Acute hepatic failure            | 23        | 48    | 71    | 4.8   |
| Postnecrotic cirrhosis           | 44        | 361   | 405   | 27.6  |
| Alcoholic cirrhosis              |           | 113   | 113   | 7.7   |
| Biliary atresias                 | 236       | 5     | 241   | 16.4  |
| Congenital hepatic fibrosis      | 6         | 4     | 10    | 0.7   |
| Cystic fibrosis                  | 3         | 4     | 7     | 0.5   |
| Inborn errors of metabolism      | 75        | 52    | 127   | 8.6   |
| Familial cholestatic syndrome    | 16        |       | 16    | 1.1   |
| Neonatal (giant cell) hepatitis  | 7         |       | 7     | 0.5   |
| Primary biliary cirrhosis        |           | 210   | 210   | 14.3  |
| Secondary biliary cirrhosis      | 9         | 13    | 22    | 1.5   |
| Primary sclerosing cholangitis   | 4         | 99    | 103   | 7.03  |
| Budd-Chiari syndrome             | 2         | 21    | 23    | 1.6   |
| Benign tumors                    | 4         | 9     | 13    | 0.9   |
| Primary liver cancer             | 8         | 59    | 67    | 4.6   |
| Bile duct cancer                 |           | 18    | 18    | 1.2   |
| Metastatic cancer                |           | 12    | 12    | 0.8   |
| Liver trauma                     | 1         | 2     | 3     | 0.2   |
| Secondary sclerosing cholangitis |           | 1     | 1     | 0.1   |
| Total                            | 438       | 1,031 | 1,469 | 100.0 |



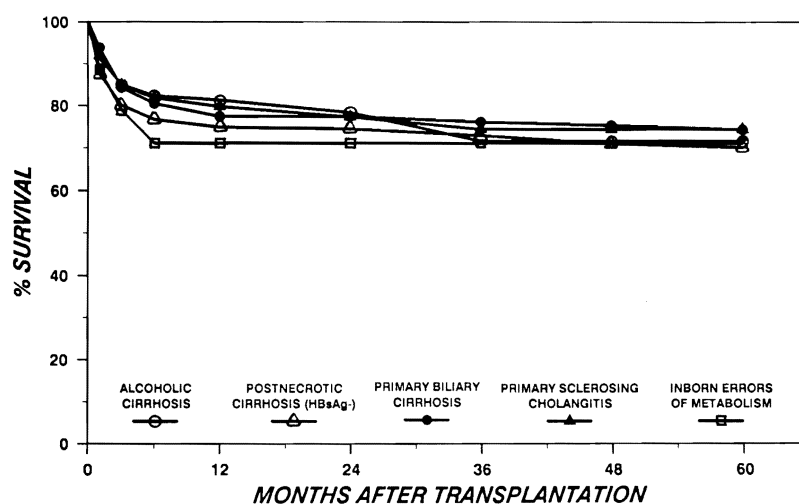
nous group of hepatic malignancies (see Table 11). The 5-year life survival curves of the principal benign adult diseases are shown in Figure 65. There has been little variability of survival with these benign diagnoses in contrast to the poorer results in the neoplastic group (see Fig 64).

More than one half of the pediatric recipients have had biliary atresia, with inborn metabolic errors a distant second.<sup>514, 516-526</sup> Survival in the biliary atresia patients is inferior to the other categories (Fig 66). The principal mortality has been perioperative and has been related to technical difficulties caused by earlier Kasai operations.

The experience reflected in these life survival curves will influence future case selection. However, other factors could be singly or cumulatively even more important for prognosis than the original diagnosis. Judgment about what constitutes candidacy has been in a state of flux since the first clinical attempts in 1963, and the time is not yet ripe to freeze guidelines.

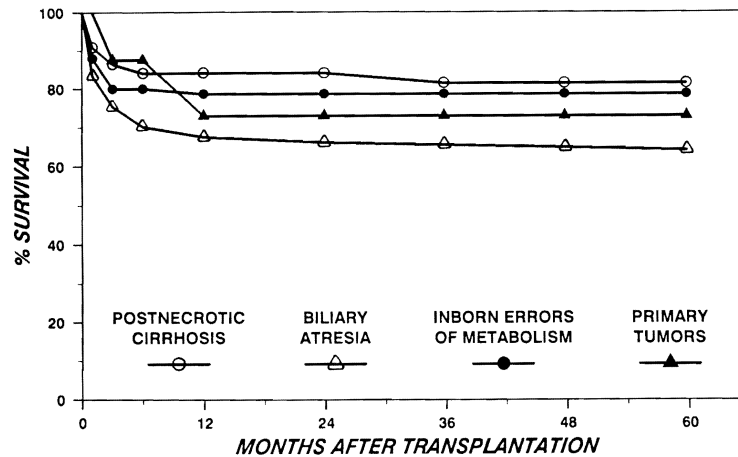
#### MALIGNANT LIVER DISEASE

In the original efforts at clinical liver transplantation,<sup>18</sup> all of the patients whose reason for transplantation was primary hepatic ma-



**FIG 65.**

Patient survival rates (life table method) after liver transplantation using cyclosporine-steroids for the major indications in adults (18 years of age or older at the time they received their first transplant). Included are 296 cases of postnecrotic cirrhosis (excluding HBsAg-positive patients), 210 cases of primary biliary cirrhosis, 113 cases of alcoholic cirrhosis, 99 cases of primary sclerosing cholangitis, and 52 cases of inborn errors of metabolism.



**FIG 66.**

Patient survival rates (life table method) after liver transplantation using cyclosporine-steroids for the major indications in children (<18 years of age when they received their first transplant). Included are 235 cases of biliary atresia, 75 cases of inborn errors of metabolism, 44 cases of postnecrotic cirrhosis, and 8 cases of primary hepatobiliary cancer.

lignancy and who survived the perioperative period died within 13 months of recurrent tumor. Smaller incidental malignancies behaved differently. The longest survivor in the world today received her new liver at the University of Colorado on January 22, 1970 for biliary atresia. The excised liver contained a 3-cm hepatoma. That little girl, 3 years old at the time of operation, will complete her 20th postoperative year in a few months. She is married to a United States Marine and lives in Okinawa. The same observations with incidental malignancies have been made many times since.<sup>186, 527</sup>

In spite of numerous disappointments, liver transplantation as a means to extend resectability limits for hepatic neoplasms is still being probed by many transplantation teams, often in combination with adjuvant chemotherapy or other experimental treatment protocols.<sup>528-530</sup> The percentage of tumor cases in large programs ranges from 4% to 34%.<sup>514, 518, 519, 531-534</sup> It has been about 5% at the Colorado-Pittsburgh program (see Table 11).

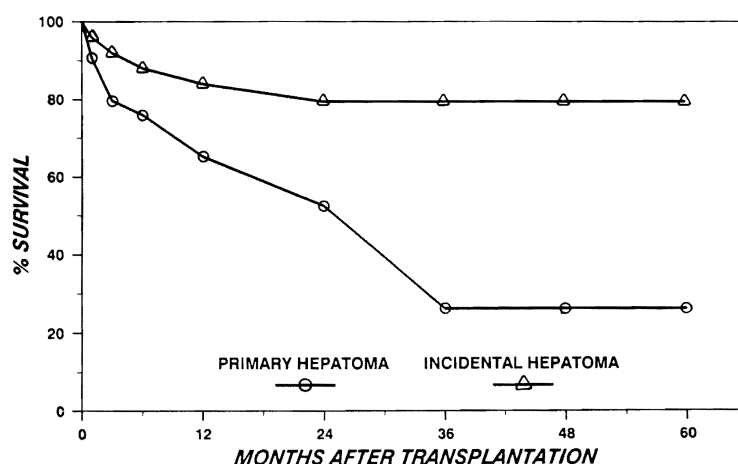
Although strenuous efforts are made beforehand to rule out metastases, a high rate of recurrence of all kinds of hepatic malignancies continues to be seen after total hepatectomy and transplantation.\* Metastases have had a tendency to home to the new liver.<sup>18, 531</sup> Death from tumor recurrence has been reported as early

\*References 18, 499, 514, 527, 531, 534-536.

as 3 months, but the principal mortality has been between 6 and 36 months (Fig 67). Small incidental malignancies that develop in cirrhotic livers usually do not recur, but extensive cancers recur in the majority of cases.<sup>527, 531, 534, 535</sup> The results also are influenced by the tumor cell type (Fig 68), presence of hilar lymph node metastases, and presence or absence of underlying liver disease.<sup>67, 499, 527, 531, 536</sup>

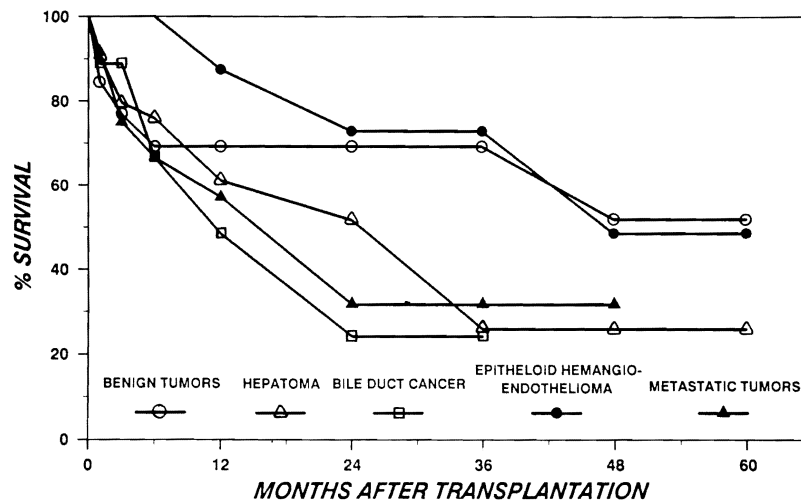
Fibrolamellar hepatoma, a slowly growing relatively uncommon hepatocellular carcinoma with distinctive histopathologic features,<sup>537, 538</sup> is a "favorable" malignancy, and long survival has been accomplished even of patients with huge tumors that have invaded the diaphragm.<sup>67, 527, 531, 534, 539</sup> Most authors have reported poor results with duct cell carcinomas, including the small Klatskin tumors that are located high in the hepatic hilum,<sup>527, 530-532, 534</sup> but a recent German experience has been more optimistic.<sup>536</sup> Recurrence has been exceptionally common in patients with conventional hepatocellular carcinomas.<sup>527</sup> Epithelioid hemangioendotheliomas<sup>540</sup> occupy an intermediary position in that survival for at least 2 years has been achieved in more than one half of reported patients.<sup>531, 541</sup>

Whether to continue treating primary hepatic malignancies is controversial. It is difficult to resist continuing these efforts for the treatment of hepatic malignancies in carefully screened recipients, not only because there is a chance of success but because there is so much potential information to be acquired about the biologic behavior of



**FIG 67.**

Patient survival rates for (life table method) after liver transplantation for primary hepatocellular cancer compared with liver transplantation for nonmalignant diseases but with an incidental hepatocellular carcinoma discovered on subsequent pathologic examination of the removed native liver.



**FIG 68.**

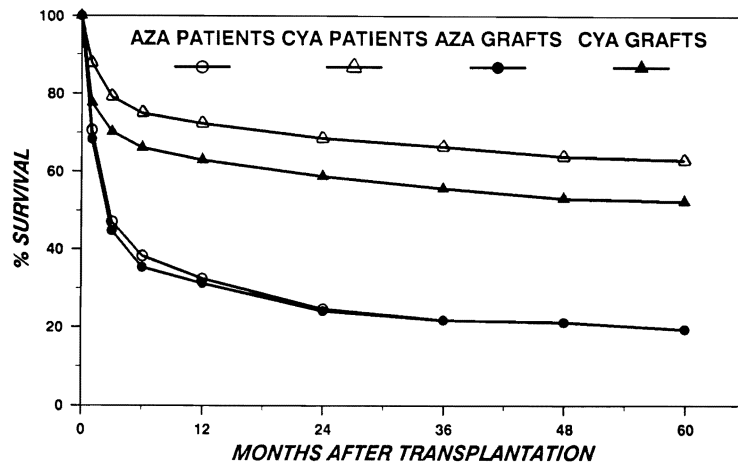
Patient survival (life table method) after liver transplantation for benign and malignant tumors that could not be treated by subtotal resection. Included are 13 patients with benign tumors, 54 with hepatocellular carcinoma, 18 with bile cancers, 8 with epithelioid hemangioendotheliomas, and 12 with secondary tumors originating outside the liver.

these tumors and the influence on them of immunomodulation and chemotherapy. Even a few patients with metastatic liver disease have benefited from liver transplantation,<sup>514, 529, 530, 535, 542, 543</sup> particularly when the primaries were neuroendocrine in origin.<sup>514, 535, 536</sup> In one remarkable case, a patient with multifocal liver metastases from a carcinoma of the breast was successfully treated with chemotherapy, autotransplantation of the bone marrow, and liver transplantation.<sup>529</sup> Ultimately, she developed recurrences; further efforts at applying this concept have failed.<sup>530</sup>

#### *BENIGN DISEASE: THE POTENTIAL CANDIDACY POOL*

The criteria for case selection were blurred until 1980 because of a mortality within the first postoperative year that exceeded 60% (Fig 69). It was impossible to tell for certain how much case selection was influencing results. When this was changed with the advent of cyclosporine (see Fig 69), some issues of candidacy became clearer.

In addition, with the better expectations and more general availability of liver transplantation, the conceptual appeal of liver transplantation was so great that this procedure became the court of last appeal for an astonishing number of patients with lethal hepatic disease. Estimates of yearly need for liver transplantation have varied



**FIG 69.**

Patient and primary graft survival rates (life table method) after liver transplantation. One hundred seventy recipients were treated with azathioprine (AZA) and steroids between March 1963 and February 1980 compared with 1,469 recipients treated with cyclosporine (CYA) and steroids between March 1980 and December 1988. Follow-up is complete through 31 July 1989.

from as low as 15 per million population<sup>67</sup> to as high as 200 per million in an unpublished Canadian projection (Dr. Cal Stiller, personal communication, University of Western Ontario, London, Ontario). Based on these figures, and without a cap imposed by organ supply, between 4,000 and 50,000 liver transplantations per year could be needed in the United States. Since there are no practical means of artificial organ support analogous to renal dialysis, the waiting list of recipients does not grow from year to year.

The variability of inclusion and exclusion factors of candidacy account for the wide-ranging estimates of need. Some of the earlier low estimates were based on the assumption that patients with tumors would be excluded, that the upper age limit would be 50 years, that patients with Laënnec's cirrhosis or other "sin factors" would be eliminated from candidacy, and that the list of applications would not be as extensive as has proved to be the case. Furthermore, a number of factors or diagnoses that precluded or strongly discouraged transplantation 5 or 10 years ago are no longer absolute contraindications, and some are no longer even questionable.

#### *Laënnec's Cirrhosis*

A prime example is alcoholic cirrhosis. If there is a history of alcoholism, it is necessary on behalf of the patient to obtain consultation

with those who understand this disease. The objective is to ensure abstinence after transplantation by arranging in advance for holistic care. In properly selected cases, Laënnec's cirrhosis may be a good indication (see Fig 65).<sup>544</sup> Recidivism with alcohol use has been less than 10%.

#### *Older Age*

An absolute upper age limit has been eliminated by demonstrating that recipients older than 50 years have a similar 5-year survival as younger adults.<sup>545</sup>

#### *Young Age or Small Size*

The transplantation of very small infants, even in the newborn period of life, has become common, but the results are not as good as with larger children.<sup>546, 547</sup>

#### *Portal Vein Thrombosis*

Although this was formerly a contraindication to transplantation,<sup>548, 549</sup> the newly developed vein graft techniques (see Fig 27) routinely allow liver replacement in recipients who have thrombosed portal, splanchnic, or superior mesenteric veins.<sup>549, 550</sup> The vein grafts are jumped from the superior mesenteric vein below the transverse mesocolon, brought anterior to the pancreas, and used for a portal anastomosis in the hepatic hilum.

#### *Multiple Previous Operations*

Previous upper abdominal operations can complicate transplantation enormously, particularly in patients with small cirrhotic livers that have extensive scarring of their inflow and outflow vessels with obliteration of potential planes of dissection. The routine measurement of liver size with imaging techniques helps to identify such problem cases in advance.<sup>551</sup> The portal vein is always studied for patency using ultrasound and dynamic computed tomography (CT) scanning techniques. In uncertain cases, magnetic resonance imaging is used. Splenectomy or any kind of shunting can alter the portal vein, and the majority of complications from transplant portal vein reconstruction have been in patients with such earlier operations.<sup>130</sup> The mesocaval and the distal splenorenal (Warren) shunts have been the least harmful of these procedures since they do not involve dissection of the portal hilum. When transplantation is performed, it is necessary to close the shunt to have optimal vascularization of the graft.

The usual indication for a shunt operation is variceal hemorrhage, and the objective is to reduce portal hypertension. Should shunting operations ever be recommended as treatment for variceal hemorrhage, knowing that these procedures can jeopardize the ultimate

step of liver transplantation? Probably uncommonly, since endoscopic sclerosis of varices is an effective alternative.<sup>552</sup> In some patients with child's class A (good risk) cirrhosis, a distal splenorenal anastomosis might be the preferred way to relieve portal hypertension. We are using this approach in a small number of highly selected patients. However, it is important to emphasize that the liver transplantation itself decompresses portal hypertension through the capillary bed of the normal new liver. In patients who had variceal bleeding and who were too sick to be considered for any operation other than transplantation, the 5-year survival after liver replacement was far superior to that reported in series of generally better-risk patients treated with shunting operation.<sup>553</sup> The obvious limitations of the shunt approach to variceal bleeding has greatly reduced the frequency of portal diversion procedures in Western countries.

Other operations in the upper abdomen that were designed to palliate complications of liver disease can create even more serious problems. Examples are procedures that disconnect venous collaterals going to lower esophageal varices and radical duct reconstructions such as those used to treat sclerosing cholangitis or biliary atresia (Kasai operation).

As an alternative to these open operations, there has been greater use of interventional radiologic or endoscopic procedures, such as sclerosis of esophageal varices, and transhepatic duct stenting or dilatation. However, problem patients with previous shunts, duct reconstructions, or other operations in the hepatic hilum should not be denied transplantation for this reason. Although the transplant operations are made more formidable, the results in experienced hands can be almost as good as with a virgin operative field.<sup>74, 554–558</sup>

### *Chronic B Virus Carrier State*

It was already mentioned that there is a very high rate of recurrent chronic active hepatitis in these patients, for which there is no effective prevention. Because of this, some programs exclude B virus carriers from candidacy. However, the fact that many such patients have achieved benefit from transplantation makes it difficult to make the carrier state an absolute contraindication.

Most efforts to treat HBsAb carriers with hyperimmune globulin (HBIG) or interferon alpha have failed.<sup>497, 498, 501, 503</sup> The volume of commercial HBIG that has been required to treat these patients has been so large as to be impractical.<sup>503</sup> However, a human monoclonal antibody directed against hepatitis B viruses has been produced (Sandoz Corporation, East Hanover, New Jersey) by fusing peripheral blood lymphocytes from an immune adult human male to a mouse × human myeloma cell line.<sup>559</sup> The resulting human monoclonal HBIG is 50,000 times more potent than commercially available

HBIGG prepared from the blood of immune donors. Seven patients were treated with this monoclonal HBIgG beginning preoperatively or at the anhepatic phase of liver transplantation.<sup>54</sup> The first recipient had reduction of surface antigen titer from very high to barely detectable levels. In the second patient, the surface antigen level was undetectable for 5 months, after which it reappeared in low titer at the same time as core antigen was identified in the hepatocytes of a biopsy specimen that otherwise was normal. The half-life of this human monoclonal IgG was long enough to allow maintenance of an antibody excess with injections 2 to 4 weeks apart.<sup>54</sup> Five patients have been treated with larger doses, and all are free of antigenemia after 2 to 7 months. It remains to be seen if the recurrent disease pattern is appreciably altered by this kind of therapy.

Recipients who possess antibodies directed against the HBV surface antigen have been free of hepatitis B virus following transplantation. However, it has been recently recorded that patients with the human immunodeficiency virus (HIV) can regress from an apparently immune state, as defined by anti-B virus antibodies, to an infectious carrier state, apparently by reactivation of residual virus as their immune system fails.<sup>560</sup> Theoretically, the same thing could occur in a liver transplant recipient maintained on standard posttransplant immunosuppression therapy.

#### *Non-A, Non-B Hepatitis*

Recurrence of non-A, non-B hepatitis<sup>144, 505</sup> has not been common. The low incidence of recurrence may merely reflect the difficulty of establishing the diagnosis.

#### *Other Recurrent Diseases*

The only other unequivocal example of disease recurrence has been with the Budd-Chiari syndrome.<sup>498, 499, 561, 562</sup> This can be prevented with anticoagulation.<sup>561, 563</sup> An initial report of recurrence of primary biliary cirrhosis<sup>564</sup> in three patients has recently been followed by an update on these patients and evaluation of 12 more primary biliary cirrhosis patients who have survived for more than 1 year. A surprising percentage<sup>565</sup> of these long-term survivors showed clinical and histologic evidence of recurrent disease. Other groups have not been able to confirm these observations in larger series,<sup>447, 566–568</sup> although the antimitochondrial antibodies usually do not disappear after transplantation or else they reappear after disappearing transiently.<sup>566, 568</sup> The reason for this discrepancy is not readily apparent, but it appears that cyclosporine may alter disease progression and histology of primary biliary cirrhosis affecting either a native liver or allograft.<sup>565</sup> Therefore, recurrences will probably not be severe or frequent enough to vitiate the value of transplantation.



A syndrome resembling sclerosing cholangitis in a liver homograft has been reported,<sup>569</sup> but the same diagnosis has been made after transplantation in patients who had non-biliary tract disease.<sup>144</sup> There has been one report of recurrent autoimmune hepatitis.<sup>570</sup>

#### *Human Immunodeficiency Virus Carrier State*

Whether patients with antibodies to HIV should be excluded from candidacy is an unresolved issue. When screening tests for this disease became generally available in the spring of 1985, examples of HIV infections in kidney recipients were almost immediately reported.<sup>571, 572</sup>

During late 1985, a massive study of the stored sera of 1,043 kidney, heart, or liver recipients treated between 1981 and 1986 was begun at the University of Pittsburgh.<sup>573</sup> Eighteen (1.7%) were found to be asymptomatic carriers. The liver recipients were most commonly affected. In about one third of the liver recipients, the HIV antibodies were demonstrated in their sera, which had been collected and stored before the transplantation. Seroconversion after liver transplantation occurred in the remaining patients, for a total incidence of 2.6%. The liver allograft itself was a source of infection in a minority of cases,<sup>573, 574</sup> and most infections were attributed to blood component therapy. Seroconversion still occurs at Pittsburgh,<sup>573</sup> as well as other institutions, despite the institution of screening enzyme immunoassays in March 1985.<sup>575, 576</sup>

Almost certainly the presence of HIV antibodies would have precluded candidacy if the diagnosis in the foregoing cases had been made in advance. As it turned out, these unfortunate victims of HIV as well as 7 additional patients became available for long-term study under immunosuppression.<sup>577</sup> Eleven of these 25 recipients were infected before transplantation, although this was not known until later in 8. The other 14 were infected perioperatively. Ten of the 25 recipients were infants or children. The organs transplanted were the liver (n = 15) and the heart or kidney (n = 5 each). After a mean follow-up of 2.75 years (range 0.7–6.6 years), 13 recipients are alive. Survival is 7 out of 15, 2 out of 5, and 4 out of 5 of the liver, heart, and kidney recipients, respectively. The best results were in the pediatric group (70% survival), in which only 1 of 10 patients died of AIDS. In contrast, AIDS caused the death of 5 of 15 adult recipients and was the leading cause of death. Transplantation plus immunosuppression appeared to shorten the AIDS-free time in HIV-positive patients compared with nontransplant hemophiliac and transfusion control groups. Accidental accrual of HIV-positive transplant recipients has slowed markedly since the systematic screening of donors, recipients, and blood products was begun in 1985. However, patients known to be HIV positive are still being treated.

It is clear that many patients can have prolonged benefit from liver transplantation in spite of having positive HIV test results. How to use this information for decision making varies from center to center. The most commonly accepted policy in the United States is to screen all recipients but not to exclude transplantation solely because of a positive HIV test result. If transplantation is undertaken, the health care personnel must be protected from infection. It is a miracle that none of the surgeons who operated on our patients in the early 1980s without knowing the risk has (to our knowledge) been infected. Screening of potential donors for HIV is obligatory at all centers, and a 50-minute test for this purpose has been described.<sup>578</sup> The use of tests that identify the HIV antigens in addition to the antibodies<sup>579</sup> may make donor screening more foolproof than it presently is.

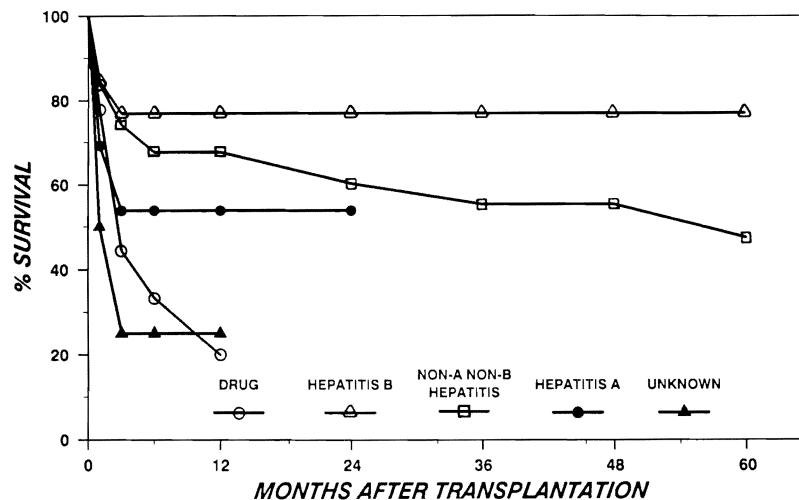
## TIMING OF TRANSPLANTATION

In the early days of liver transplantation, this therapeutic step seemed so drastic that it was used as a last resort. What was then defensible conservatism has become regressive today if the patient is allowed to deteriorate to the point of requiring life support systems before thinking of the transplant option. The rapidity of this deterioration is highly variable.

### *FULMINANT HEPATIC FAILURE*

The diagnosis of fulminant hepatic failure (FHF) can be made when there is sudden massive necrosis of a liver that previously has functioned normally.<sup>580-582</sup> The term FHF has not been used for acute exacerbation of previously unrecognized chronic disease or for acute Wilson's disease. It was rarely treated with liver transplantation before 1982.<sup>67</sup> The results with transplantation has not been good enough to justify this drastic step for a disease syndrome from which recovery might occur in 5% to 20% of cases.<sup>580-582</sup> Since then, FHF has been accepted as an emergency indication for transplantation in almost every liver transplant program worldwide. In several large series,<sup>583-591</sup> the predominant diagnoses have been non-A, non-B hepatitis, B virus hepatitis, and toxic hepatitis from a variety of agents. Mushroom poisoning has been a much publicized toxic etiology.<sup>592</sup> In our hands, the original diagnosis has strongly influenced the outcome (Fig 70). The best results have been with B virus hepatitis.

A decision to proceed with liver replacement often must be made in a few hours. The systematic collation of multiple parameters can help distinguish patients who have a good chance of recovery from those who will die without transplantation.<sup>593, 594</sup> The etiology of the FHF may be an important prognostic determinant.<sup>594</sup> Premonitors of imminent death include relentless progression over a 7- to 14-day period, grade 3 or 4 encephalopathy, severe coagulopathy, rapid shrinkage of the liver as documented with imaging techniques, metabolic acidosis, cardiovascular instability, and sepsis.<sup>585, 586</sup> By the



**FIG 70.**

Patient survival (life table method) after liver transplantation in adults and children for fulminant hepatic failure. Included are 9 cases of drug-related liver failure, 13 cases of acute B virus hepatitis, 31 cases of acute non-A, non-B hepatitis, 13 cases of acute hepatitis A, and 4 cases of fulminant hepatic failure of unknown etiology.

time there is grade 4 encephalopathy and ventilator dependence, it usually is too late.

If transplantation is performed before these grave findings, some livers with reversible lesions may be replaced unnecessarily. A liver biopsy after correction of the coagulopathy may provide decisive information. If clotting cannot be corrected well enough to permit a closed needle biopsy, the patient can be explored with a new liver in hand with the option of aborting the operation if the open biopsy looks favorable histopathologically. In spite of the pitfalls associated with liver replacement for FHF, current posttransplant survival rates of 55% to 75%<sup>583-591</sup> compare favorably with the most optimistic projections of 20% for medical management alone. The results make it certain that these efforts will continue. The perioperative mortality frequently has been due to brain stem herniation during or just after transplantation, sometimes in spite of continuous monitoring of intracranial pressure. Early referral to liver transplant centers, extremely aggressive evaluation plus medical treatment, and an early decision for surgical exploration with immediate transplantation as an option will be necessary to improve results.

It will be unfortunate if the availability of transplantation causes the therapeutic pendulum to swing too far toward liver replacement.

In the hepatology unit at King's College, London, the admission of patients with FHF to an intensive care unit, the continuous monitoring of intracranial pressure, and attention to multiple details has resulted in greatly improved survival (more than 50%) of patients whose survival expectation in the past would have been less than 20%.<sup>594</sup> They emphasize the value of IV mannitol treatment as a means of brain shrinkage and hypoventilation on respirator control to encourage cerebrovascular vasodilatation by keeping the  $P_{CO_2}$  elevated.<sup>594</sup>

Similarly, Levy Sinclair and associates of Toronto have reported the astonishing recovery of patients (10 or 17) with FHF.<sup>595</sup> Some of their patients had liver biopsies in which it was difficult to find a single living hepatocyte. They ascribed their success to prostaglandin E, namely, Prostin, a synthetic prostaglandin that can be given intravenously or orally. In their opinion, an important, and possibly the principal, value of Prostin was to preserve the integrity of the hepatic microvasculature and thus to ensure a viable scaffold on which regeneration could proceed.

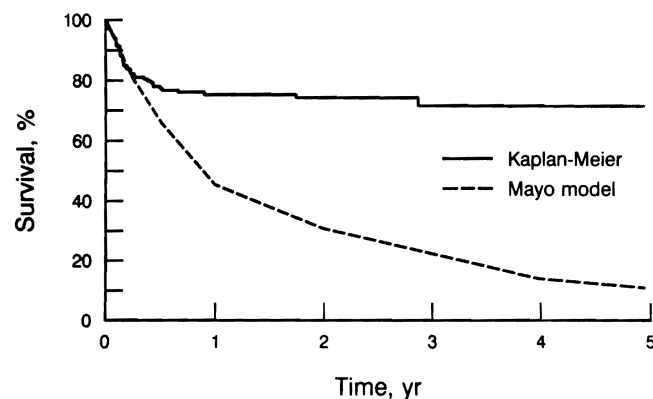
#### *END-STAGE CHRONIC DISEASE*

Ideally, a candidate for liver replacement should have an unequivocal need for transplantation but still be well enough to participate in the complex process of recovery. A decision to go forward requires input from the primary physician, who may see gradually evolving and often appalling social and vocational invalidism that may not be evident at first examination. The disability may be reflected in the loss of intellectual capacity with encephalopathic dementia, frequent hospitalizations for other complications of liver failure, inability to function in a domestic environment, and arrest of growth and development in infants and children. These issues of quality of life loom large in most patients long before the truly terminal events of chronic hepatic failure. Formulas for candidacy based on liver function tests have not been helpful because the abnormalities in these tests are so variable from disease to disease or even within the same disease. Patients with cholestatic disorders (e.g., biliary atresia and primary biliary cirrhosis) usually become deeply jaundiced with good preservation of hepatic synthetic functions for a long time,<sup>520, 525, 557</sup> whereas patients with hepatocellular disease may not be jaundiced in spite of the most profound depressions in albumin and prothrombin synthesis.<sup>548</sup>

The liability of procrastinating too long before making a decision for transplantation has yet to be defined. In one study in which 12% of candidates died "while waiting," most of the lost patients had arrived at the transplant hospital on ventilators and had GI bleeding,

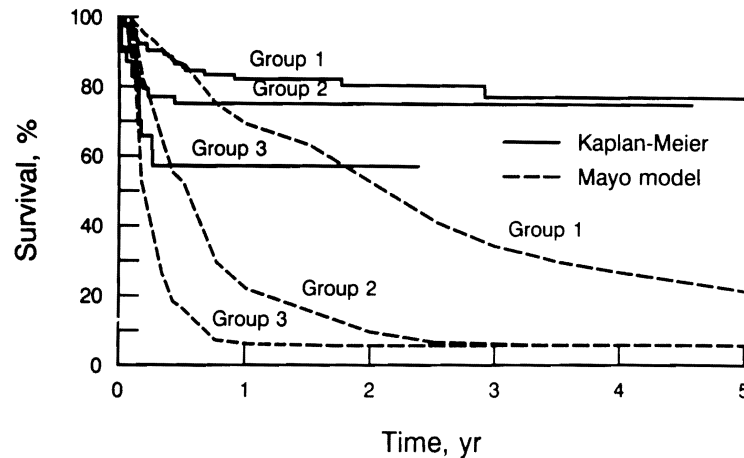
coagulopathies, the hepatorenal syndrome, aspiration pneumonitis, subacute bacterial peritonitis, or other end-stage complications.<sup>596</sup> In another center, the mortality in patients considered too well to be placed on the active waiting list was greater than for those admitted to candidacy.<sup>597</sup> When the mistake of underestimating disease severity with the supervention of a catastrophic complication is made, resuscitation is sometimes successful. However, the outlook after subsequent transplantation is demonstrably degraded,<sup>598, 599</sup> notwithstanding observations in a small group of pediatric liver recipients that disease severity did not seem to influence posttransplantation prognosis.<sup>600</sup>

The most precise studies of disease staging vs. posttransplantation outcome have been in adult patients with primary biliary cirrhosis.<sup>601, 602</sup> In the most recent of these investigations,<sup>601</sup> disease severity was defined with a formula in which age, serum bilirubin level, serum albumin level, prothrombin time, and edema severity accurately predicted life expectancy without transplantation.<sup>603</sup> The overall survival in transplant recipients was greatly improved relative to these predictions (Fig 71). However, the patients who were still in reasonable condition had a low perioperative mortality and a 2-year survival of 80%; those with the most serious deterioration had a high perioperative mortality and a 2-year survival of only 55% (Fig 72).<sup>601</sup> The consensus in most centers is that transplantation should be considered at an earlier time before the stage of catastrophic complications is reached.<sup>604</sup>



**FIG 71.**

Comparison of the projected survival in patients with primary biliary cirrhosis when treated with transplantation (Kaplan-Meier) vs. the expected outcome with all alternative forms of treatment (Mayo model). (From Markus BH, Dickson ER, Grambsch PM, et al: *N Engl J Med* 1989; 320:1709–1713. Used by permission.)



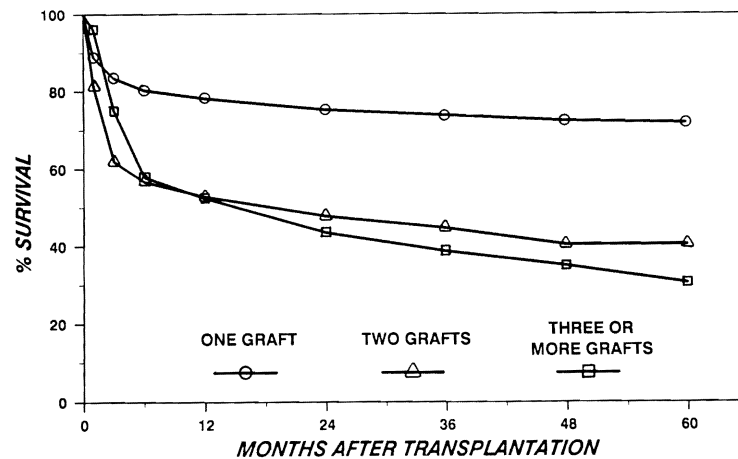
**FIG 72.**

The influence of disease severity on the projected survival vs. the survival achieved with transplantation. Group 1 patients were in the best condition and group 3 patients in the worst. The prognosis without transplantation was worse in all stratifications, but so were the results after transplantation. This study quantified the penalty of undue procrastination before referral and treatment of patients with this disease. (From Markus BH, Dickson ER, Grambsch PM, et al: *N Engl J Med* 1989; 320:1709–1713. Used by permission.)

Recently, an increasing number of patients with normal liver function and nonmalignant hepatic masses have had orthotopic transplantation for polycystic disease,<sup>217, 219</sup> cystic hygroma,<sup>605</sup> and adenomatosis. The size of those lesions and the consequent disability and life-threatening complications of the mass lesions were the indications for operation. The largest of the excised livers weighed 16.5 kg.<sup>605</sup>

#### THE QUESTION OF RETRANSPLANTATION

Before the advent of cyclosporine, retransplantation was a rare event. Consequently, the graft and patient survival were almost synonymous (see Fig 69). Almost immediately after the introduction of cyclosporine, attempts at retransplantation began to be made and with enough success to warrant further such efforts.<sup>67</sup> Now the patient survival curves began to be 10% to 15% above the graft survival curves (see Fig 69). In the United States at the present time, approximately one fifth of all liver grafts are used for retransplantation. The need for retransplantation is often extremely urgent, and many patients have a clinical syndrome comparable with or worse than fulminant hepatic failure.



**FIG 73.**

Survival of patients who required only one graft (1,125 cases) is significantly better ( $p < 0.001$ ) than for patients requiring two transplants (268 cases) or three or more transplants (76 patients).

The success rate with retransplantation is only about one half of that if a primary graft succeeds (Fig 73). The chances of 5-year survival with a "take" of the first graft is about 75% (see Fig 73), almost twice as good as the expectation if two or more grafts are needed. This low success rate with retransplantation has caused ethicists to question the probity of continuing these efforts. Yet, the salvage of so many patients whose first grafts have failed seems more than adequate justification for what has been done.

If the option of retransplantation was foreclosed, it would have a chilling effect on donor acceptance since the philosophy of one chance only would discourage the transplantation of grafts with more than minimal preservation times and would greatly tighten the requirements for donor consideration. No liver transplant surgeon of whom we are aware would countenance the concept of patient abandonment implicit in a policy that precludes or even discourages retransplantation in a patient who is potentially salvageable.



## INBORN ERRORS OF METABOLISM: A PANDORA'S BOX

Patients with liver-based inborn errors of metabolism can be treated by providing a phenotypically normal liver.<sup>237, 464, 606–639</sup> It was recognized long ago and confirmed repeatedly since that the  $\alpha$ -globulins, haptoglobin,<sup>237, 464</sup> and group-specific component,<sup>464</sup> as well as other products of hepatic synthesis,<sup>640–645</sup> permanently retain the original metabolic specificity of the donor after transplantation. These observations made it virtually certain that liver transplantation would become a decisive way to treat the inborn errors of metabolism that resulted partly or completely from deficiencies of specific liver enzymes or from abnormal products of hepatic synthesis. This expectation has been fulfilled in many patients for whom follow-ups of as long as 18 years after transplantation are available (Table 12). With other disorders in which the pathogenesis was not well understood, the transplantation itself became a powerful research tool by showing the extent of correction and by elucidating the mechanisms by which correction was accomplished (see Table 12). In one patient, the opposite of a therapeutic correction was achieved in that a coagulation defect present in the donor was conferred on the recipient.<sup>646</sup>

In the majority of these recipients, the inborn error had itself been responsible for damage to the liver, and a conventional indication of liver failure or the development of malignant tumors prompted the liver replacement. In these cases, the correction of the metabolic error was incidental. However, an increasing number of transplantations have been carried out solely for the purpose of correcting the inborn error, and in many of these latter patients (see Table 12), the excised liver has been anatomically normal.

Many inborn errors not correctable by liver transplantation can be effectively treated with allogeneic bone marrow engraftment.<sup>647</sup> Determining which kind of transplantation will be effective is crucial whenever somatic metabolic engineering is considered. The guidelines for decision making have become increasingly clear.<sup>54, 647</sup>

**TABLE 12.**

Inborn Errors Treated With Liver Transplantation

| Disease                            | Explanation of Disease   | Correction of Metabolic Defect | Longest Survival | Associated Liver Disease           | Reference   |
|------------------------------------|--|--------------------------------|------------------|------------------------------------|-------------|
| $\alpha_1$ -Antitrypsin deficiency | Structural abnormality of the protease inhibitor synthesized in liver  | Yes                            | 13 yr*           | Cirrhosis                          | 606–609     |
| Wilson's disease                   | Abnormal biliary copper excretion, decreased copper binding to ceruloplasmin, and copper accumulation in tissues; autosomal recessive gene mapped to chromosome 13 | Yes                            | 16.5 yr*         | Cirrhosis                          | 606,610–616 |
| Tyrosinemia                        | Fumaroylacetoacetate hydrolase deficiency  | Nearly complete                | 7.5 yr*          | Cirrhosis, hepatoma                | 617–619     |
| Type I glycogen storage disease    | Glucose-6-phosphatase deficiency   | Yes                            | 7 yr*            | Glycogen storage, fibrosis, tumors | 620         |
| Type IV glycogen storage disease   | Amylo-1: 4,1: 6-transglucosidase (branching enzyme) defect   | Incomplete†                    | 4.5 yr*          | Cirrhosis                          | 606,612     |
| Cystic fibrosis                    | Unknown; pancreatic disease, liver often affected  | Not known                      | 4.5 yr*          | Cirrhosis                          | 621,622     |
| Niemann-Pick disease               | Sphingomyelinase deficiency, sphingomyelin storage   | Not known                      | 2 yr (died)      | None                               | 623         |
| Sea-blue histiocyte syndrome       | Unknown, neurovisceral lipochrome storage  | No                             | 7 yr*            | Cirrhosis                          | 624         |
| Erythropoietic protoporphyria      | Hepatic ferrochelatase deficiency, ?overproductive of protoporphyrin by erythropoietic tissues   | Incomplete                     | 1.5 yr           | Cirrhosis                          | 625,626     |

|                               |   |            |          |  |         |
|-------------------------------|---|------------|----------|--|---------|
| Crigler-Najjar syndrome       | Glucuronyl transferase deficiency   | Yes        | 4 yr     | None   | 627-628 |
| Type 1                        |   |            |          |  |         |
| hyperoxaluria                 | Peroxisomal alanine: glyoxylate aminotransferase deficiency                         | Yes        | 8 mo.    | None   | 629     |
| Urea cycle enzyme deficiency  | Ornithine carbamoyltransferase deficiency   | Yes        | 8 mo.*   | None   | 630     |
| C protein deficiency          | Defective C protein synthesis   | Yes        | 2.25 yr* | None   | 631     |
| Familial hypercholesterolemia | Low-density lipoprotein receptor deficiency, low-density lipoprotein overproduction | Incomplete | 6 yr*    | None   | 632-635 |
| Hemophilia A                  | Factor VIII deficiency  | Yes        | 4 yr*    | Cirrhosis, a complication of blood component therapy | 636-638 |
| Hemophilia B                  | Factor IX deficiency  | Yes        | 6 mo.    | Cirrhosis, a complication of blood component therapy | 639     |

\*Patients in University of Colorado—University of Pittsburgh series. Follow-up to January 1989.

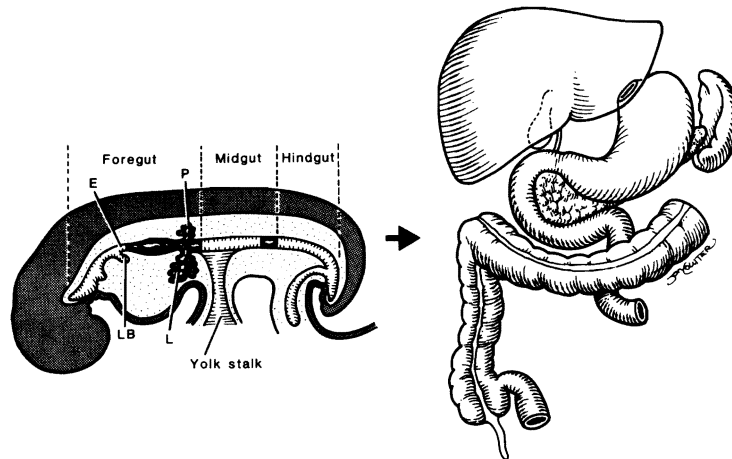
†Amyloid deposits found in heart biopsy 4 yr after transplantation.



# TRANSPLANTATION OF MULTIPLE ORGANS

The increasing boldness with which hepatic transplantation has been applied is evident from the many reports of transplantation of the liver plus kidney<sup>217, 219, 648–651</sup> and less frequently used combinations of the liver plus pancreas,<sup>281</sup> liver plus heart,<sup>632–635, 652</sup> and liver plus heart and lung.<sup>653</sup> In these cases, the liver transplantation and transplantation of the other organ have been done in discontinuity so that two standard procedures were performed in the same individual.

A different concept has been the inclusion of the liver in visceral organ clusters. The most complex operation of this kind has been of the liver and pancreas plus the entire GI tract in two children with the short-gut syndrome and secondary liver failure that developed during parenteral hyperalimentation.<sup>654, 655</sup> One of these grafts (Fig



**FIG 74.**

**Left**, delineation in embryonal life of that region of the GI tract (*dark shaded*) that was resected in the organ cluster operation (*E* = esophagus; *LB* = lung bud; *L* = liver; *P* = pancreas). **Right**, the adult organs deriving from the shaded primitive analogue. (From Starzl TE, Todo S, Tzakis A: *Ann Surg* 1989; 210:374–386. Used by permission.)

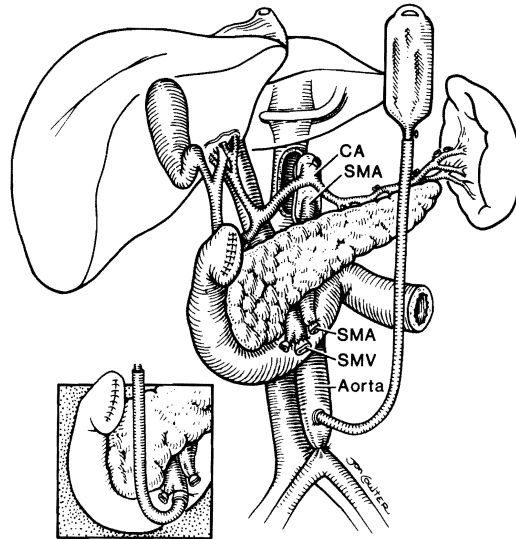
74) provided function of all of the organs for more than 6 months before the recipient died of complications of lymphoproliferative tumors in the liver.<sup>654</sup> With an organ mass of this size, the possibility of carrier lymphoid tissue causing GVH disease was feared. In the longest surviving patient, donor pretreatment with OKT3 may have reduced this threat,<sup>654</sup> as has been demonstrated to occur with anti-lymphocyte serum in rats.<sup>656</sup>

A less drastic version of multivisceral transplantation is the use of an organ cluster in which the pancreas, duodenum, and part of the proximal jejunum have been included with the liver.<sup>54, 657</sup> These clusters have been used to replace upper abdominal organs that were removed (see Fig 74) in treating sarcomas and carcinoid tu-



**FIG 75.**

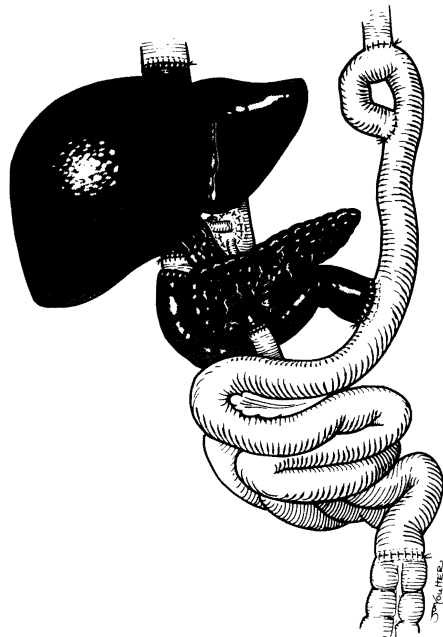
The CT scan (*top*) of patient whose upper abdomen was filled with spindle cell sarcoma at the time of operation. The tumor-laden liver is the structure to the left of the operating room photograph (*bottom*). Most of the right half of the diaphragm was removed with the specimen. The transverse colon is marked with *white arrows*. The margins were free of tumor, and none of the 38 lymph nodes studied had metastases. (From Starzl TE, Todo S, Tzakis A: *Ann Surg* 1989; 210:374–386. Used by permission.)



**FIG 76.**

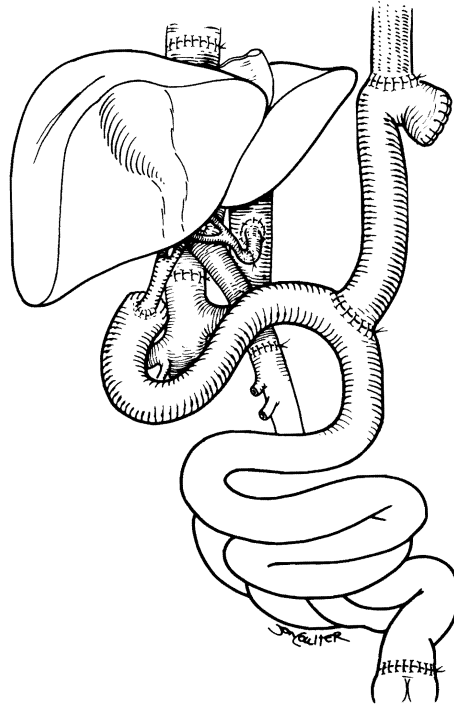
Removal of organ cluster graft from donor. The specimen is initially cooled with an aortic infusion of UW solution after crossclamping the proximal abdominal aorta. Once the specimen has been removed with a Carrel patch containing the origin of the celiac axis (CA) and superior mesenteric artery (SMA), the liver is secondarily perfused on the back table with UW solution (*insert*) through the superior mesenteric vein (SMV). (From Starzl TE, Todo S, Tzakis A: *Ann Surg* 1989; 210:374–386. Used by permission.)

mors of the pancreas or duodenum with liver metastases (Fig 75), bile duct carcinomas with liver metastases, and a hepatoma that had invaded the duodenum and colon.<sup>657</sup> The organs removed from the recipient in continuity have included the liver, stomach, pancreas,



**FIG 77.**

Completed reconstruction in the recipient. (From Starzl TE, Todo S, Tzakis A: *Ann Surg* 1989; 210:374–386. Used by permission.)



**FIG 78.**

This is an alternative to the reconstruction after an upper abdominal exenteration in which only the liver is replaced. This operation leaves the patient diabetic, but of 15 patients treated in this way, 13 are alive with follow-ups of several weeks to as long as 6 months. (From Tzakis A, Todo S, Starzl TE: *Transplant Proc* February 1990 [in press]. Used by permission.)

spleen, duodenum, proximal jejunum, and ascending plus transverse colon (see Fig 74). The organs transplanted are shown in Figure 76. The completed recipient operation is shown in Figure 77.

Of 15 such patients, 9 are alive after 6 to 14 months, 8 without evidence of recurrent tumor. The ninth survivor may have stable pulmonary metastases. The majority of the survivors have been rehabilitated. This experience has illustrated how major components of the GI tract can be transplanted and has demonstrated how the use of organ clusters can allow extirpative procedures of a magnitude not previously imaginable.

The major limitations of the cluster operation have been the difficulty of finding appropriate organ donors, the difficulty of the operation, and the complexity of postoperative care. Considering the fact that of the organs being replaced, only the liver is indispensable, an



alternative was developed in which the same resection was performed but only the liver was transplanted (Fig 78). Fifteen such patients have been so treated, but the follow-ups are too short to merit comment. This variation of the original cluster procedure has been developed as a more pragmatic operation but at the expense of rendering the patient apancreatic. Malabsorption has been a serious clinical problem thus far, and thus it may influence cyclosporine doses. The day-to-day treatment of diabetes mellitus has not been difficult. If management of the iatrogenic diabetes mellitus proves difficult, pancreas transplantation at a more favorable moment remains an option.



## QUALITY OF LIFE

Even in the early days of liver transplantation, the physical and emotional decay caused by chronic liver disease could be stopped and reversed in many of the recipients who survived chronically. The most powerful determinants of their quality of life were the liver function profile at the 1-year convalescent mark and the quantity of steroids needed to maintain this function.<sup>658</sup> The adverse steroid factor in the quality of posttransplant life has been reduced since the introduction of cyclosporine. Several studies have shown the remarkable restoration of physical and emotional well-being that can be expected in infants and children,<sup>658-660</sup> including resumption of growth or even catch-up growth.<sup>661</sup>

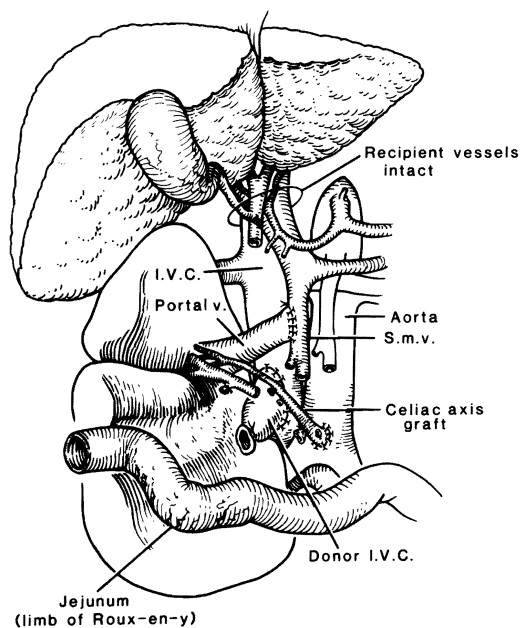
Similarly, a recent group of adult liver transplant recipients studied objectively before and again 2 years after operation demonstrated broad improvement in social interaction, home management, alertness, the utilization of recreation and leisure time, and overall psychosocial functioning.<sup>662</sup> A number of other findings were obtained from these investigations. First, the severity of stress experienced by the patient and the spouse after transplantation correlated significantly with the ease of recovery. More than 90% of the recipients who had a single transplantation state that they have no problems or only minor health problems 2 years after transplantation. More than 85% have returned to work and state that they are able to perform their jobs well. In contrast, the smaller number who required more than one transplant had a much poorer outcome, with only 43% being able to work because of one or more disabilities.

The follow-up of patients treated in the cyclosporine era dates back to only 1980. However, a bellwether group of survivors remains from an original series of 170 patients treated from 1963 to 1979.<sup>67, 663</sup> Twenty-eight of these recipients are still living after 10 to 19 years. These represented exactly one half of the survivors at 1 year. Only two patients who were alive at 5 years died subsequently. One of the late deaths was caused by chronic rejection 12.5 years after retransplantation. The other death was from a lymphoma after 13.5 years. Rehabilitation has been complete in the long survivors.<sup>663</sup>



## THE OPTION OF AUXILIARY TRANSPLANTATION

With the auxiliary operation, as originally described in unmodified dogs,<sup>1</sup> the extra liver was placed in the right paravertebral gutter, reararterialized from convenient adjacent vessels, and provided with a portal venous inflow with systemic blood from the recipient iliac



**FIG 79.**

This is the kind of auxiliary liver transplantation that has permitted several long-term successes. Note that the graft receives a portal flow from the splanchnic venous system (S.M.V.) and is drained into the inferior vena cava (I.V.C.). The principles of this operation were originally worked out by Marchioro and colleagues.<sup>18</sup> (From Starzl TE [with the assistance of Putnam CW]: *Experience in Hepatic Transplantation*. Philadelphia, WB Saunders Co, 1969. Used by permission.)

vein or lower vena cava. The graft outflow was drained into the recipient inferior vena cava. It was observed that auxiliary grafts were much more severely damaged than were orthotopically placed livers, primarily because of rapid hepatocyte atrophy.<sup>664</sup> These adverse effects could be prevented by diverting splanchnic venous flow through the auxiliary liver and away from the recipient's own liver,<sup>665</sup> suggesting that the splanchnic venous blood contained specific liver-supporting factors. The most important of these so-called portal hepatrophic substances was proved to be insulin.<sup>325, 666</sup>

The condition of providing a splanchnic venous inflow to the graft has been met in almost all of the subsequent clinical trials, which by 1978 numbered more than 50 (Fig 79).<sup>667</sup> Auxiliary liver transplantation with unquestionable prolongation of life was first achieved at the New York Memorial Hospital on December 13, 1972.<sup>668</sup> The recipient, who had biliary atresia, still is alive with a follow-up of more than 16 years.<sup>669</sup> In 1980, Houssin and associates in Paris reported a 29-month survival of an adult who was given an extra liver.<sup>670</sup> This patient was HBsAg-positive and died 8 years following transplantation from a hepatocellular carcinoma in his host liver (H. Bismuth, personal communication, January 1989).

With the increased success of orthotopic liver transplantation, interest in auxiliary transplantation waned. Very few further efforts were reported in the last decade.<sup>671</sup> The resulting pessimism has been lightened by a recent report of the transplantation of whole livers or liver fragments to the right paravertebral gutter of six adult recipients using essentially the same operation as that tried in earlier times.<sup>672</sup> At the time of reporting with follow-ups of 5 to 23 months, all six recipients were alive. Cautious further trials undoubtedly will be forthcoming.

## PRACTICAL LIMITATIONS

### *ORGAN SUPPLY*

Organ supply increasingly will influence candidacy criteria. However, discussions about rationing transplant services for this reason are premature since the balance between the need and supply of livers has not been determined. In the United States, the yearly rate of liver transplantations has reached approximately 1,600,<sup>673</sup> averaging 147 per month between July and December 1988 (Dr. William Vaughn, United Network of Organ Sharing, personal communication, 1989). The annual European total is approaching this figure.<sup>674</sup>

Policies about organ donation will have to be reexamined if substantial further growth is to occur. Probably, many potential liver donors are being rejected for inappropriate reasons. The arbitrary upper age limit for liver donors observed by most programs<sup>675</sup> cannot be justified since the liver is the only organ that does not undergo senescence.<sup>676</sup> Atherosclerosis of its arterial supply usually is not found beyond the origin of the celiac axis.<sup>676</sup> A limited experience with livers from donors older than 50 years has been encouraging.<sup>677</sup>

Other potential donors of all ages often are excluded because of poor blood gases, a need for inotropic or vasopressor drugs, minor abnormalities of liver function test results, or the existence of other diseases such as diabetes mellitus.<sup>675</sup> The results with such donors both in the United States<sup>161, 162</sup> and Europe<sup>163</sup> have been as good as with so-called perfect donors. The use of better preservation techniques<sup>51-53</sup> that allow safe storage of liver grafts for 1 day instead of the previous 6 or 8 hours should reduce organ wastage, since with this extra time, countrywide and worldwide networks of organ sharing can be set up.

### *ECONOMIC FACTORS*

The ability to pay for liver transplantation has had a profound influence on candidacy. Ironically, the feasibility first and then the practicality of liver transplantation were established without considering how to finance this revolutionary form of therapy. In 1983, a planning commission for the state of Massachusetts estimated the average cost of liver transplantation in the first year would be \$238,000,<sup>678</sup> although the actual costs were only one third this high

in a large program already in existence.<sup>114</sup> It is clear that astronomical bills can be generated if patients are too disabled by the time of transplantation, if the first liver graft does not function well, and if serious complications develop, including the need for retransplantation.<sup>114</sup>

Because of their fear of runaway expenses, many health insurance carriers and government agencies have avoided financial responsibility to their constituents by classifying liver transplantation as "experimental"<sup>679</sup> in spite of the Consensus Development Conference conclusion to the contrary. The response to cost-conscious funding agencies is that liver transplantation can eliminate repeated and expensive hospitalization of patients who are slowly dying with chronic hepatic disease.<sup>680-682</sup> Such considerations were part of a bitter controversy in Australia<sup>683, 684</sup> about the establishment of what eventually proved to be two outstanding programs.<sup>685, 686</sup>

So far, liver transplantation in the United States has been paid for by a heterogeneous system of private health care insurance programs, government agencies, and public or private fund-raising activities. One highly visible consequence has been the recurrent spectacle of a family or patient pleading on television or through other media for economic support or for an organ. All the while, statistics that show gross underparticipation in this new kind of health care by blacks and presumably other disadvantaged groups have been accruing.<sup>687</sup> Development of a system that allows all citizens equal and reasonable access to this kind of treatment without the extraordinary expenses of past programs such as the federally financed End Stage Renal Disease program may require new and creative administrative approaches.



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